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CHEMICAL SYNTHESIS OF PHENOXATHIN ANALOGS OF ACTINOMYCIN

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CHEMICAL SYNTHESIS OF PHENOXATHIIN ANALOGS OF ACTINOMYCIN

BY

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B. S., Boston College, 1958

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Paul R. Jones

December 16, 1965
Date

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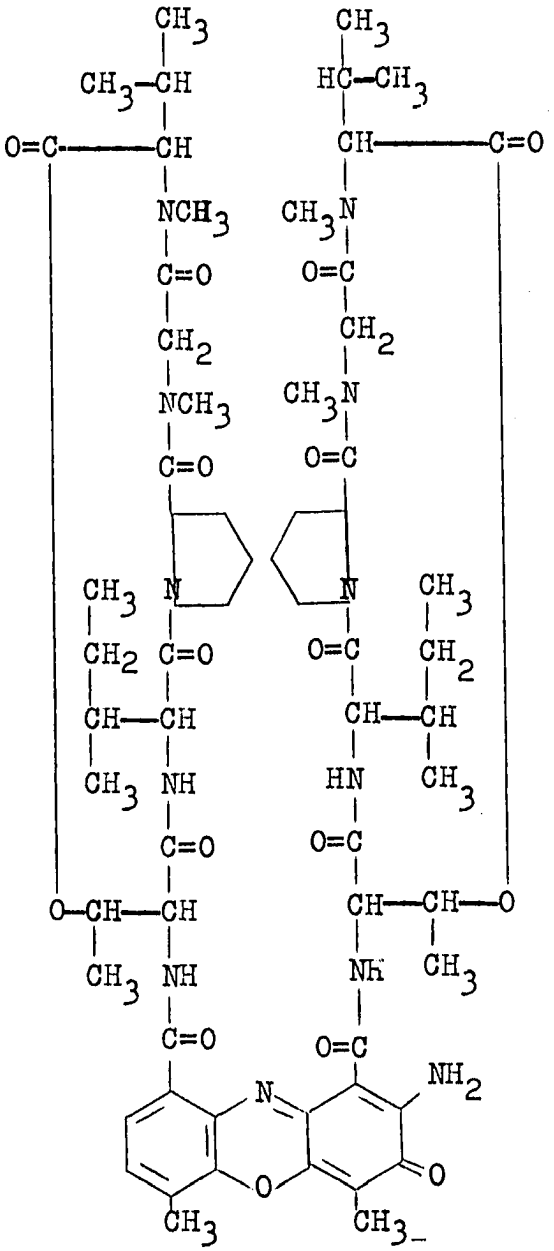
INTRODUCTION AND HISTORICAL

In 1940, Waksman (1) succeeded in isolating the first true antibiotic produced by Actinomyces (now Streptomyces) antibioticus. This antibiotic was called actinomycin A. It was crystallized as a red-pigmented substance possessing high activity against many Gram-positive bacteria; but, it was somewhat less active against fungi and Gram-negative bacteria. To experimental animals the antibiotic was highly toxic, doses of 0.5 to 1.0 mg./kg. of body weight being lethal. However, the recognition of the cytostatic properties of the actinomycins aroused interest in their potential anticancer activities (2).

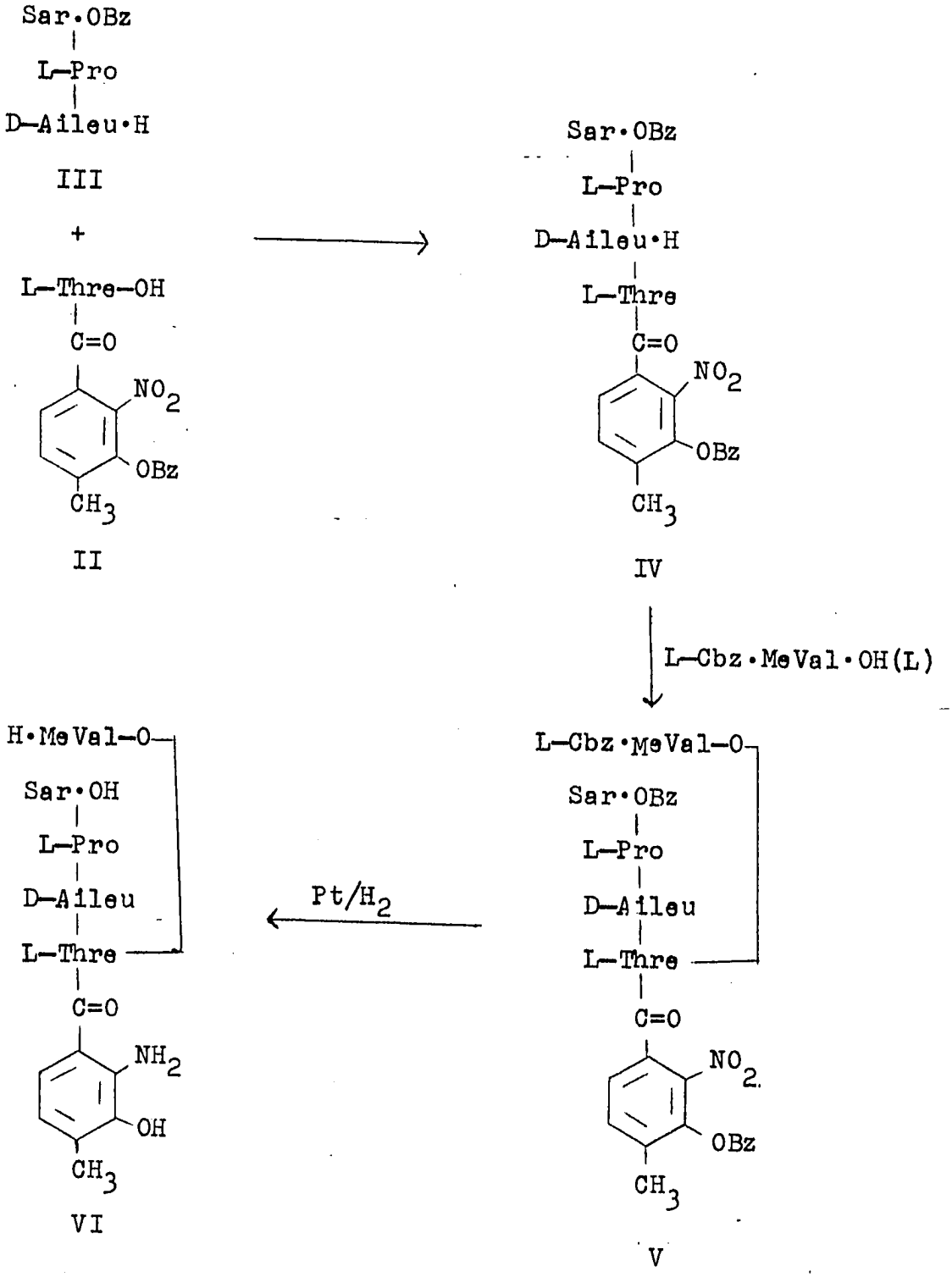
Since the discovery by Waksman (1), various actinomycins have been produced. An example is the actinomycin C mixture isolated from Streptomyces chrysomallus (3). Brockmann has shown that the mixture consisted of three different actinomycins (C_1, C_2, and C_3), which differed only in the polypeptide portion of the molecule (4,5).

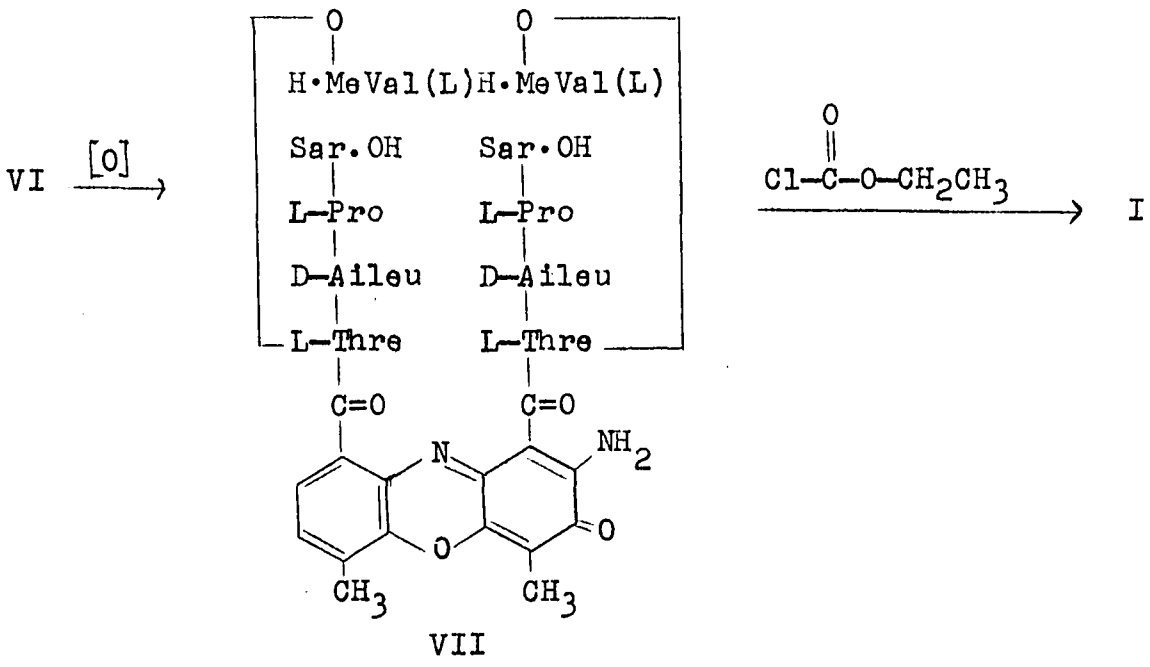
The elucidation of the structures of the actinomycins was started by Brockmann on actinomycin C_3, and in 1956 he and his coworkers established via a degradation scheme that the structure, I, of this antibiotic contained the chromophore of 2-amino-4,6-dimethyl-3-oxo-phenoxazine-1,9-dicarboxylic acid which was attached by an amide linkage to a polypeptide, L-threonyl-D-alloisoleucyl-L-prolylsarcosyl-L-N-methylvaline, cyclized by lactone formation between the

terminal carboxyl group of valine and the hydroxyl group of the threonyl fragment. This structural assignment was substantiated by its successful synthesis (5).



I



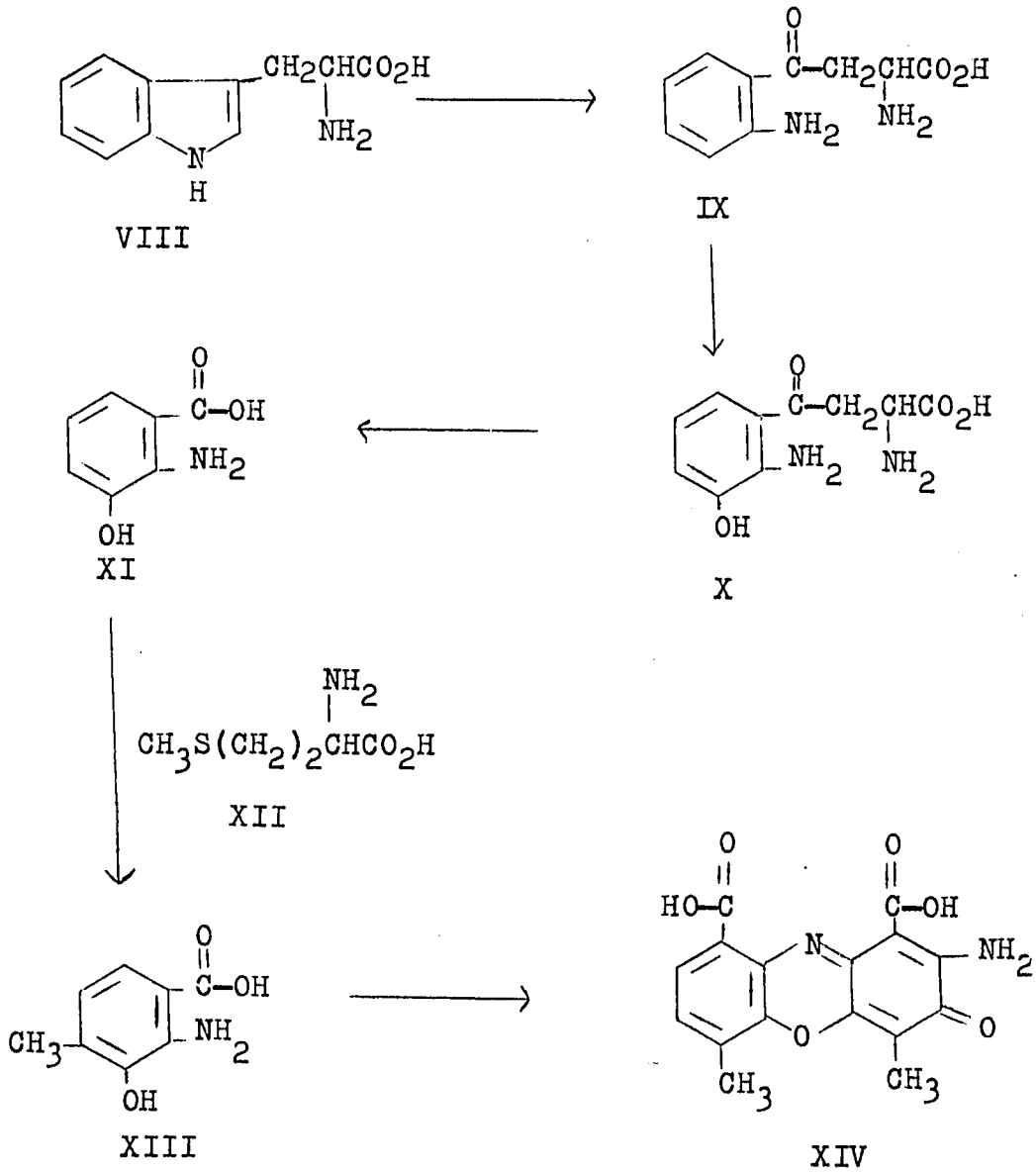


VII

In 1956, Schmidt-Kastner (6) introduced sarcosine to a Streptomyces strain which normally gave only actinomycin C_3. Now actinomycin C plus several new actinomycins in which sarcosine was incorporated at abnormal positions in the polypeptide portion of the molecule were produced. Several abnormal amino acids have also been incorporated into the polypeptide portion of actinomycins by introducing the abnormal amino acid directly into the culture medium. In none of these cases, however, can a control of the relative position of introduction of the amino acids be achieved.

The biosynthetic origin of the heterocyclic portion of actinomycin was suggested by Katz (7) to be tryptophan (VIII). He postulated that Streptomyces antibioticus metabolized tryptophan(VIII) via kynurenine(IX) and 3-hydroxykynurenine(X) to 3-hydroxyanthranilic acid(XI), which

then undergoes methylation by methionine(XII) at the 4-position to yield the 3-hydroxy-4-methylanthranilic acid (XIII). As suggested by Brockmann (5), XIII was postulated to undergo self condensation to give the 2-amino-4,6-dimethyl-3-oxo-phenoxazine-1,9-dicarboxylic acid(XIV).



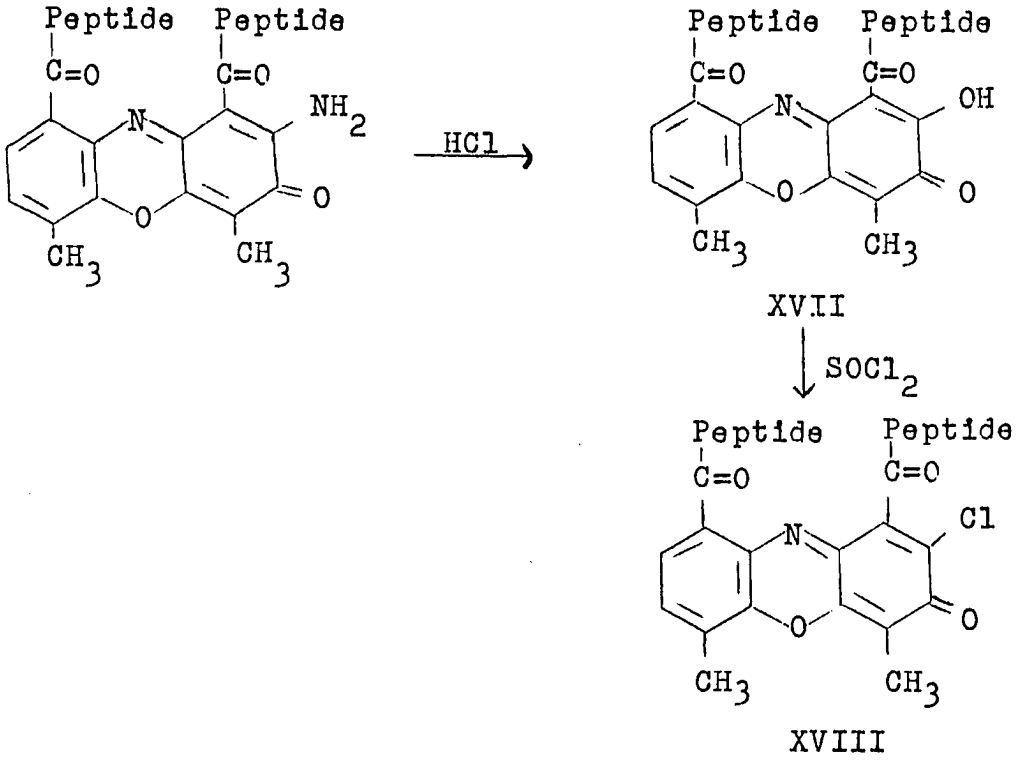
From studies on the biological mechanism of action of actinomycin D (10,11,12), it was concluded that actinomycins having appropriate structural features bind tightly, but reversibly, to DNA. The bound DNA can no longer serve as a template for the synthesis of RNA, thereby terminating RNA and consequently, protein syntheses.

Clinically, the most publicized of the actinomycins, actinomycin D, (replacement of two molecules of D-alloisoleucine by two molecules of L-valine in Structure I), was found to cause regression of two childhood neoplasms, Wilms' tumor and embryonal rhabdomyosarcoma, and also to show modest effects in Hodgkin's disease (9).

The chemical and pharmacological interest in actinomycins suggested the importance of a project of syntheses of compounds containing the two types of systems found in the actinomycins, that is, a heterocyclic nucleus and a polypeptide system. Consequently, since their discovery, much interest has been shown in the synthesis of actinomycin analogs in an attempt to reduce the toxicity, while retaining the antibiotic activity.

Brockmann prepared the first analogs of actinomycins by modification of the substituents on the heterocyclic ring in the natural product by chemical means. For example, replacement of the 2-amino group with chloride by the reaction

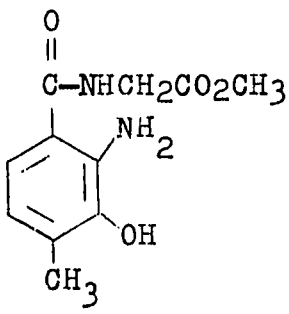
of the natural product with hydrochloric acid gave the desamino actinomycin(XVII). XVII on reaction with thionyl chloride was converted to the chloro actinomycin, XVIII.



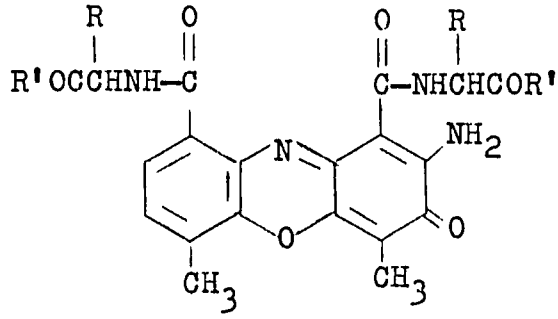
XVIII

Modification of the heterocyclic portion of actinomycins would be expected to be difficult by biological synthesis since the bacteria would probably not incorporate new heterocyclic acids into the antibiotic molecule. Furthermore, as has been pointed out, a control of the relative position of introduction of the amino acid units cannot be achieved by biological synthesis. Therefore, it appeared that a general method of synthesis of actinomycin analogs would require a totally chemical method of preparation if the analogs were not to be synthesized from the natural product. The importance of this approach has been evidenced

by several publications. Brockmann (8) prepared the first true analog (XVIa) of the actinomycins by the air oxidation of an aqueous solution of methyl 2-amino-3-hydroxy-4-methylhippurate(XV) in ammonium carbonate. Mauger (23) reported the synthesis of actinocinyl-bis-glycine ethyl ester(XVIb) and of some derivatives of 3-benzoyloxy-4-methyl-2-nitrobenzoyl-L-threonine(II) by the method of Brockmann (6). Weinstein (13) prepared actinocinyl-bis-L-threonine(XVIc), its dimethyl ester (XVId), and its diamide (XVIe) by oxidative procedures also similar to that reported by Brockmann (6).



XV



XVI a, R=H; R'OH

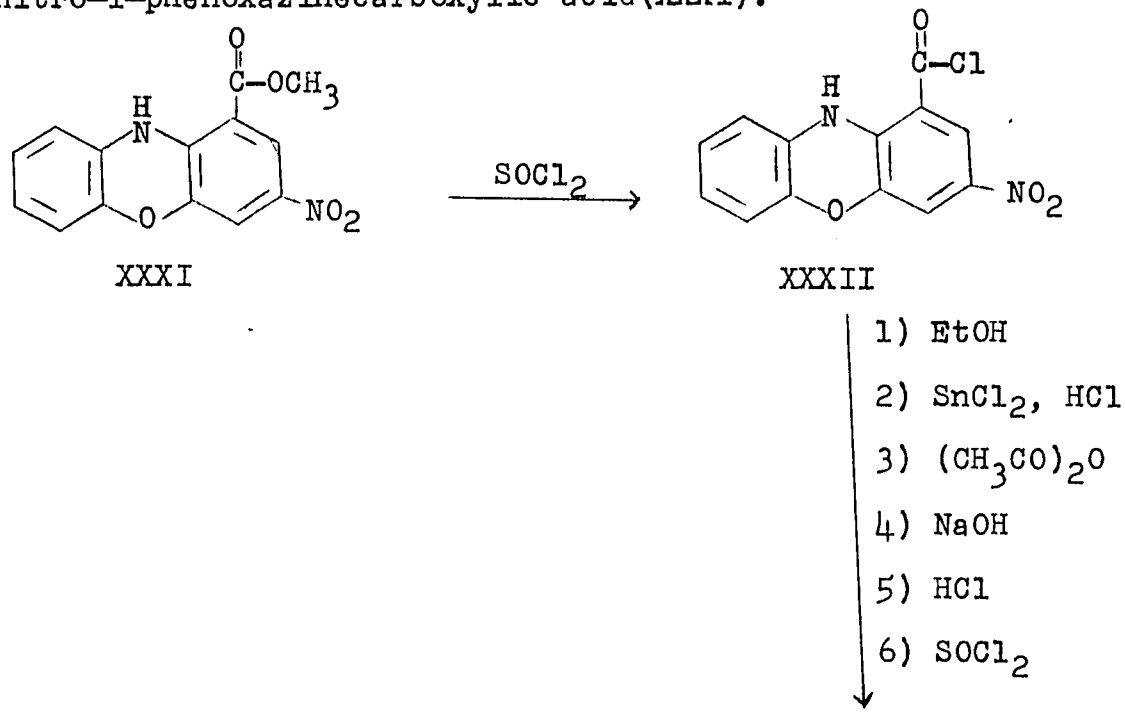
b, R=H; R'=OCH_2CH_3

c, R=$CH_3CH(OH)$; R=OH

d, R=$CH_3CH(OH)$; R'=OCH_3

e, R=$CH_3CH(OH)$; R'=NH_2

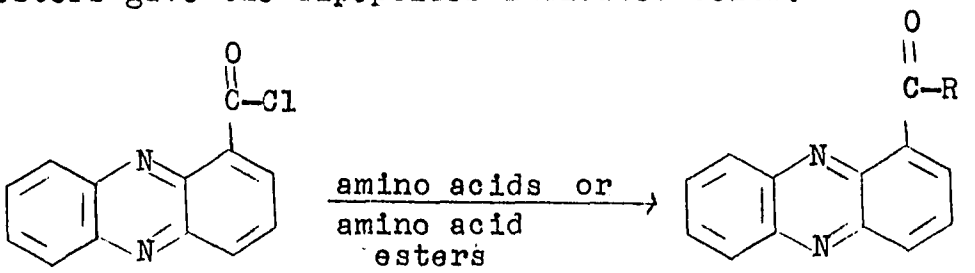
Predroditeleva (20) prepared several derivatives of substituted 1-phenoxazinecarboxylic acid, starting with 3-nitro-1-phenoxazinecarboxylic acid(XXXI).



XXXI XXXII XXXIII XXIV

Peptide derivatives having phenazine as the heterocyclic nucleus were prepared by Yoshioka (21). Starting with 1-phenazinecarboxylic acid chloride(XXXV) and an amino acid or amino acid ester, the monopeptides indicated below were synthesized. In order to prepare dipeptide derivatives, the glycine derivative was converted to the azide, XXXVI,

by reaction with sodium nitrite in acetic acid. The azide XXXVI, on reaction with various amino acids or amino acid esters gave the dipeptides indicated below.



XXXV

R=$NHCH_2CO_2CH_3$

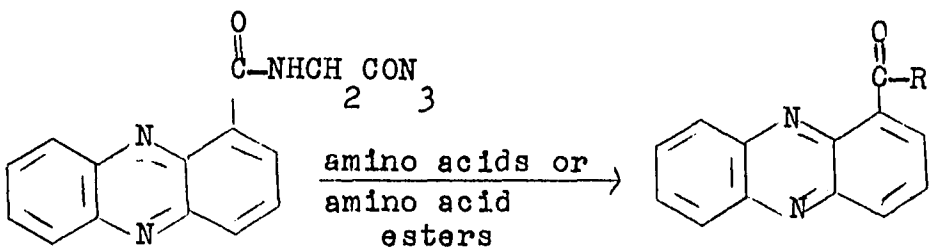
=$NHCH_2CO_2H$

=$NHCH(CH_3)CO_2H$

=$NHCH(CH_2CH_3)CO_2H$

=$NHCH\left[CH(CH_3)_2\right]CO_2H$

=$NHCH\left[CH_2CH(CH_3)_2\right]CO_2H$



XXXVI

R=$NHCH_2CONHCH_2CO_2CH_2CH_3$

= $NHCH_2CONHCH_2CO_2H$

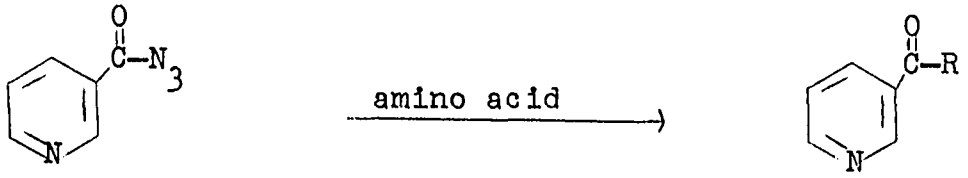
=$NHCH_2CONHCH(CH_3)CO_2H$

=$NHCH_2CONHCH(CH_3)CO_2CH_3$

=$NHCH_2CONHCH(CH_2CH_3)CO_2CH_2CH_3$

=$NHCH_2CONHCH(CH_2OH)CO_2CH_3$

Wu (22) synthesized several actinomycin analogs with nicotinic acid as the heterocyclic moiety. The use of the azide, anhydride, chloride and p-nitrophenyl ester of nicotinic acid for activation of the carbonyl for amide formation was investigated. The most successful general reagent for these preparations was found to be nicotinyl azide (XXXVII). The results of this study are summarized below.



R=$NHCH_2CO_2H$

=DL–$NHCH(CH_3)CO_2H$

=L–$NHCH(CH_3)CO_2H$

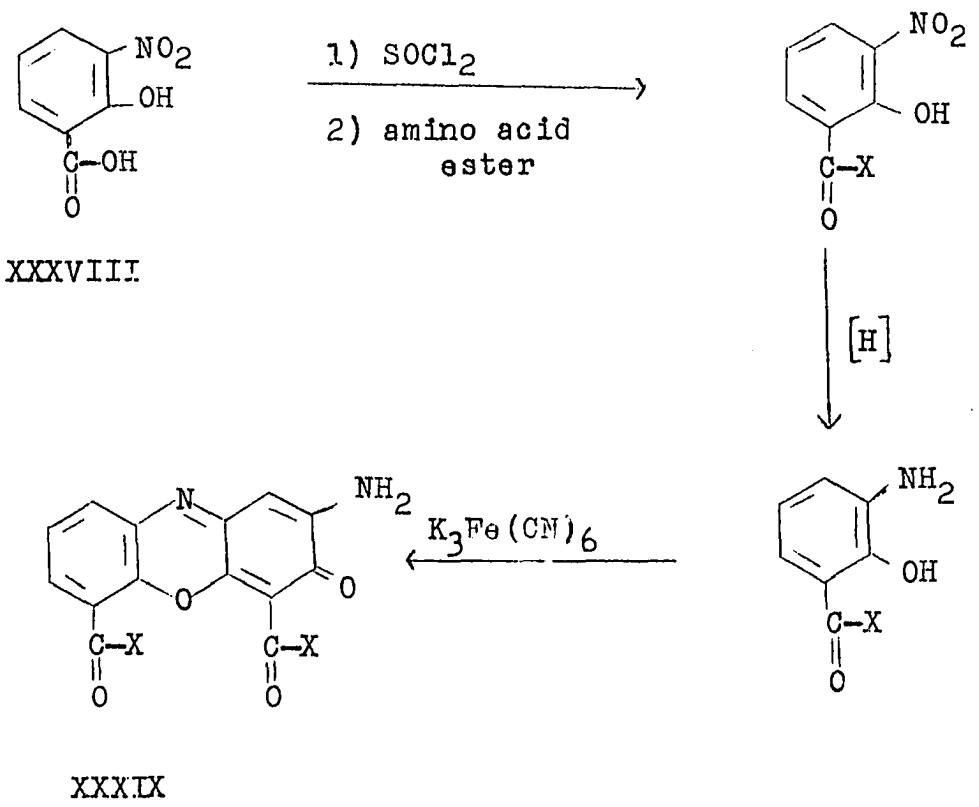
=DL–$NHCH[CH(CH_3)_2]CO_2H$

=L–$NHCH[CH_2CH(CH_3)_2]CO_2H$

=DL–$NHCH(CH_2C_6H_5)CO_2H$

=o–$NH_2C_6H_5CO_2H$

Using 3-nitrosalicylic acid(XXXVIII) as a starting material, Wu (22) also prepared a number of amino acid ester derivatives of 2-amino-3-oxo-phenoxazine-4,6-dicarboxylic acid(XXXIX), in which the physiological activity was greater than that of the nicotinic acid derivatives listed above.



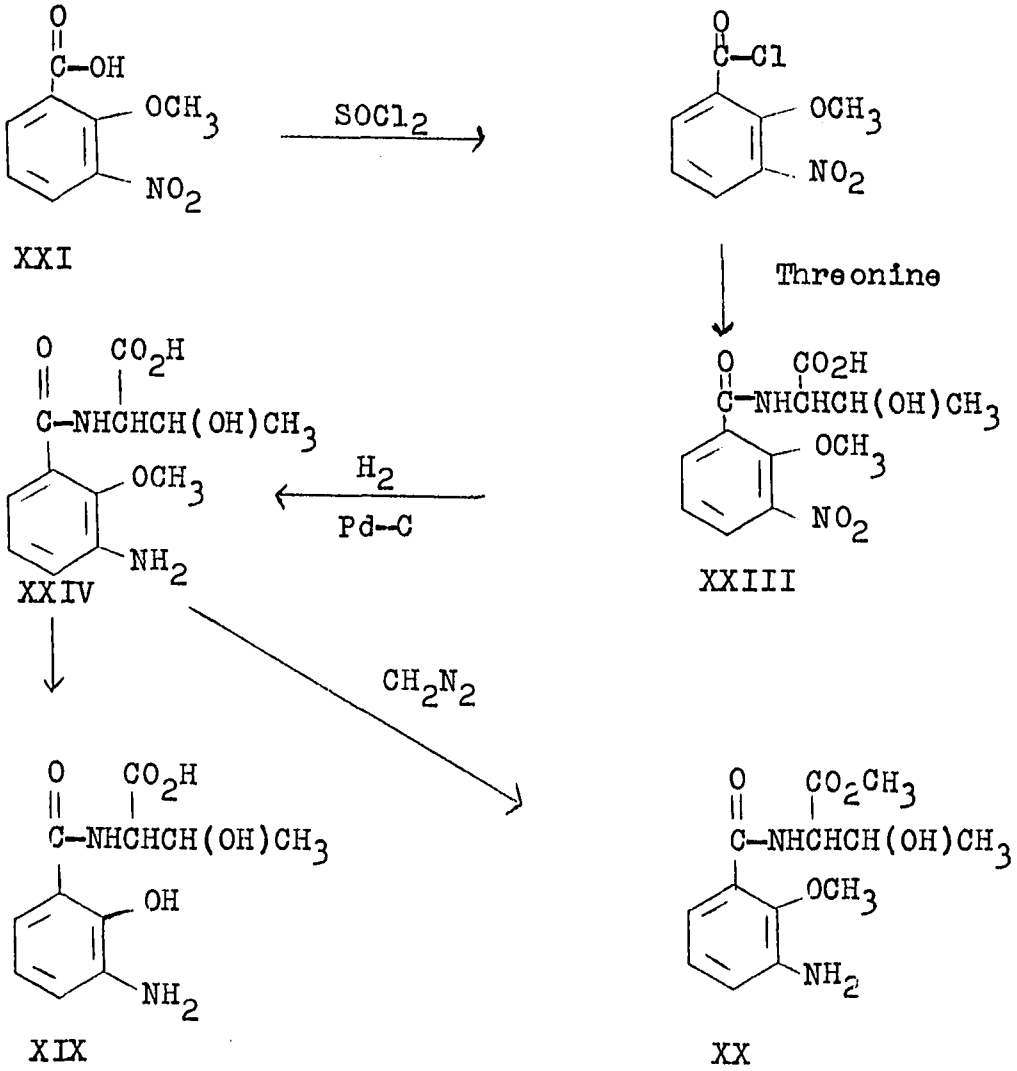
XXXVIII

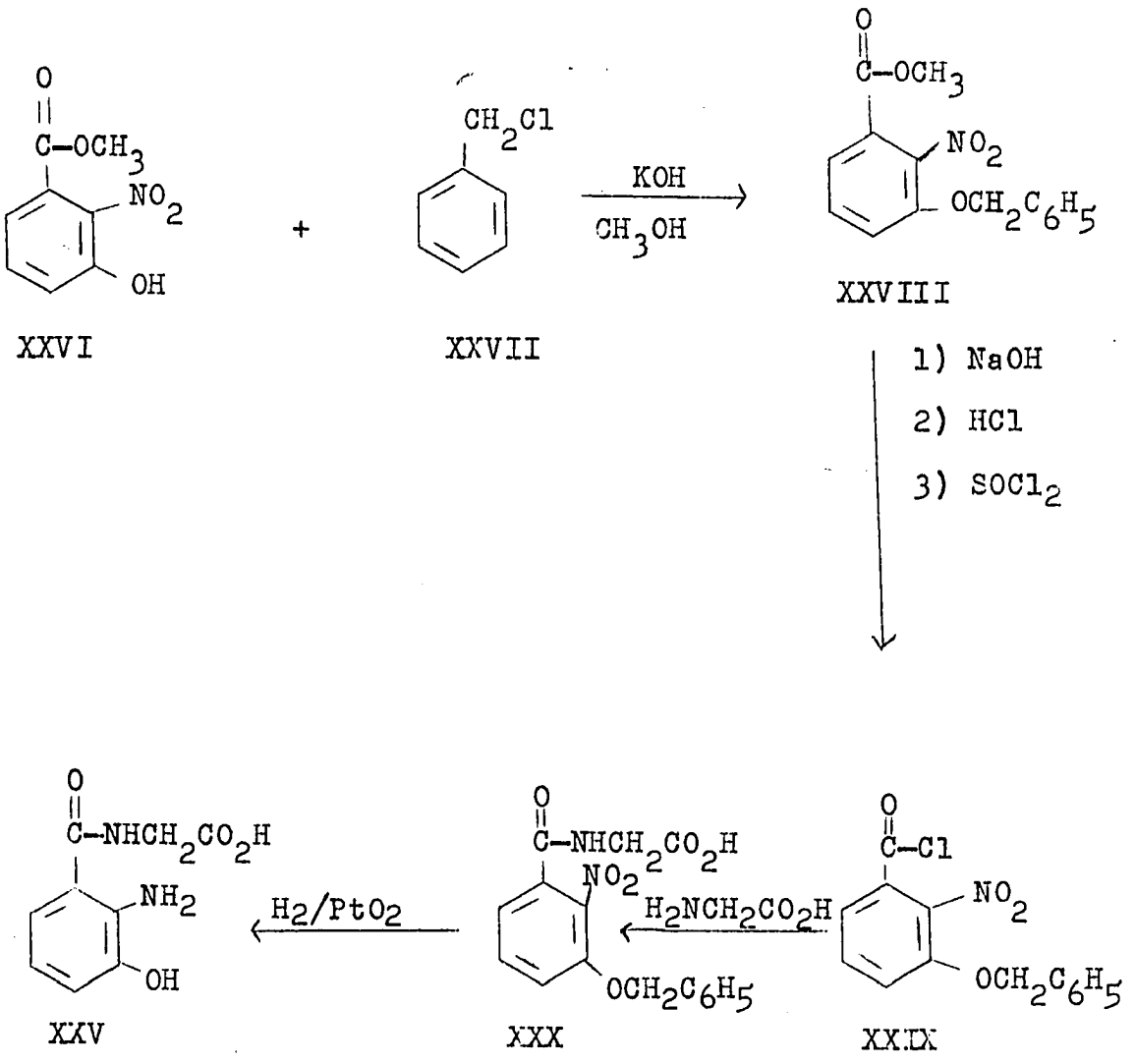
XXXIX

As has been demonstrated above, the approach to the chemical synthesis of actinomycin analogs usually required the preparation of a heterocyclic acid. Various derivatives, especially the amino acid or amino acid ester derivatives, of the heterocyclic acid were then usually prepared in order to investigate their chemotherapeutic values.

The syntheses of several analogs of the nonheterocyclic precursor of actinomycin have been reported, and their pharmacological activity has been compared with that of analogs having a heterocyclic nucleus. O. Rumura (15, 16) prepared antimycin acid (XIX), which was isolated by Dunshee (17) from an unidentified species of Streptomyces,

and its methyl ester (XX). Senoh (19) synthesized N-(3-hydroxyanthraniloyl)glycine(XXV), which was found in excreta of white-2-mutant silkworm larvae in which the conversion of 3-hydroxykynurenine(X) to pigment is blocked. The low degree of pharmacological activity of XIX, XX, and XXV, support the idea that a heterocyclic molecule attached to the peptide chain might be the reason for the observed high degree of activity in a number of actinomycin analogs having a heterocyclic nucleus.





The interesting chemotherapeutic properties of the complex heteroaroyl polypeptide, actinomycin, suggested the possibility of potential activity for some other tricyclic, heteroaroyl system. In choosing a tricyclic, heterocyclic system for the synthesis of actinomycin analogs, certain characteristics were sought: (1) the starting material should be available commercially; (2) the ring system should be found in some known pharmacologically active compounds;

(3) derivatives of this heterocyclic compound analogous to actinomycin should be novel. These characteristics are satisfied by phenoxathiin(L) since, (1) phenoxathiin(L) is available from Eastman Organic Chemicals. (2) Phenoxathiin (L) and several of its derivatives have been shown to be bacteriostatic toward streptococci, to possess insecticidal properties, and to be anthelmintics (27,34,35). (3) The amino acid and polypeptide derivatives of phenoxathiin(L) would be novel.

Actinomycin possesses amide carbonyl groups located at the two peri-positions ortho to the heterocyclic nitrogen atom, as was shown in structure I. It appeared that a compound possessing carboxylic acid groups in the two peri-positions ortho to the heteroatom (O or S) of phenoxathiin (L) would provide the closest analog in structure to the heteroaroyl portion of actinomycin.

The electrophilic substitution of phenoxathiin(L) normally leads to the 2-substituted and 2,8-disubstituted derivatives which would not be suitable in the preparation of the desired dicarboxylic acid. The possible synthesis of the dicarboxylic acid by oxidation of the corresponding 4,6- or 1,9-dialkyl phenoxathiin was not feasible since the starting materials were not readily available and oxidation would have affected the sulfur. A possible route to the desired dicarboxylic acid appeared to be via metalation. The ability of this reaction to yield ortho products, usually unmixed with para isomers, distinguishes metalation from the

more familiar types of electrophilic substitution and makes it possible to prepare many products not readily available by other routes.

Gilman (41) reported the synthesis of 4,6-phenoxathiindicarboxylic acid(LXVIIa) in a 34% yield from the dimetalation of phenoxathiin(L) with n-butyllithium(XLI). An improvement of his isolation procedures could lead to the satisfactory application of this route to the desired dicarboxylic acid. If, however, this dimetalation approach failed to give a substantial yield of the 4,6-phenoxathiindicarboxylic acid(LXVIIa), then metalation of its derivatives would be investigated in an attempt to find a route to a 4,6-disubstituted phenoxathiin which could be converted to the desired dicarboxylic acid.

Gilman (24) reported that the electron availability of the heteroatom was important in directing the attack of the n-butyllithium(XLI) to a position ortho to a heterocyclic atom. He also reported that the directing influence of oxygen was more important than that of sulfur. Using these observations of Gilman, a possible alternate route to 4,6-phenoxathiindicarboxylic acid(LXVIIIa) was the metalation of 4-hydroxymethylphenoxathiin(LXX), formed by reduction of 4-phenoxathiincarboxylic acid(LII). The electron density on the phenoxathiin ring would be increased due to the electron donating ability of the 4-hydroxymethyl group. If the directing influence of oxygen still prevails in LXX, then a metalation reaction of this carbinol (LXX)

would be expected to yield 4-hydroxymethyl-6-phenoxathiin-carboxylic acid(LXXI). LXXI could then be oxidized by standard procedures to 4,6-phenoxathiindicarboxylic acid (LXVIIa).

Once this heteroaroyl analog of actinomycin(LXVIIa) was prepared, the synthesis of its amide derivatives would be attempted in order to prepare analogs more closely related in structure to actinomycin. It was expected that these derivatives could be readily prepared by a Schotten-Baumann procedure.

FIGURE 1

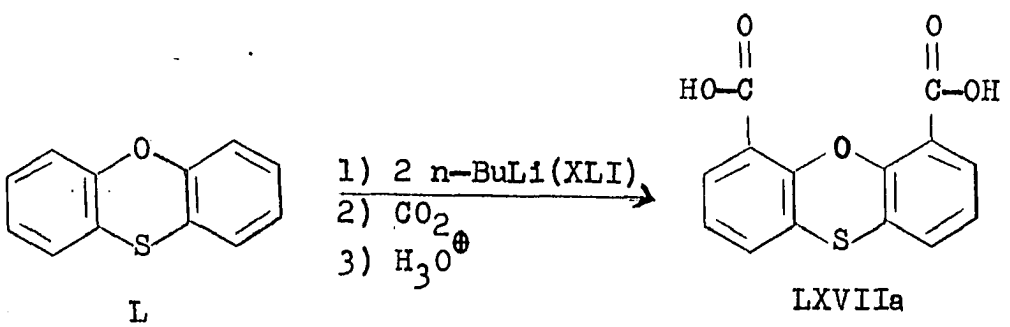
PROPOSED REACTION SCHEME FOR THE SYNTHESIS

OF PHENOXATHIIN(L) ANALOGS

OF ACTINOMYCIN

1) Synthesis of 4,6-phenoxathiindicarboxylic acid(LXVIIa).

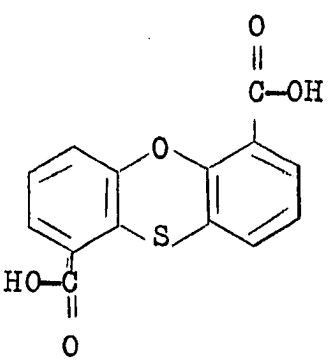
Procedure A



L

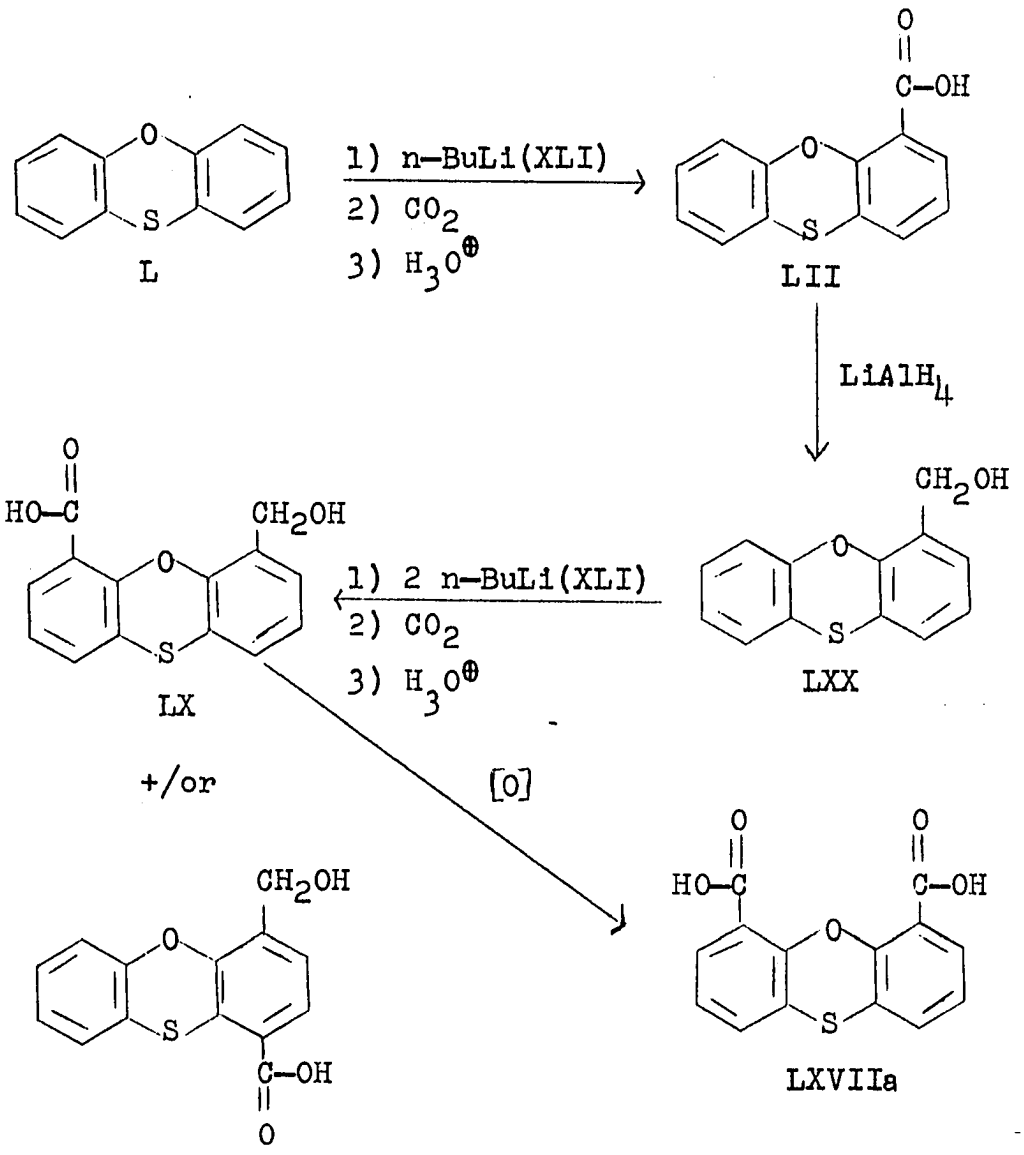
LXVIIa

+

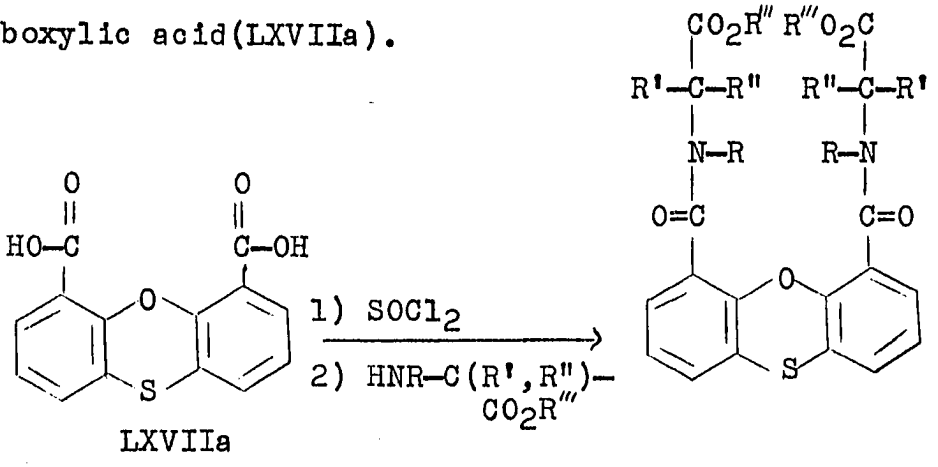


LXVIIIa

Procedure B



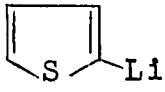
2) Synthesis of peptide derivatives of 4,6-phenoxathiindicarboxylic acid(LXVIIa).



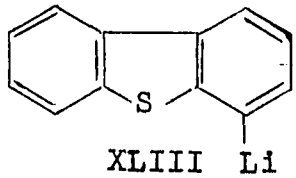
DISCUSSION

Analogs of actinomycin having a phenoxathiin ring were desired. As described in the Introduction, the synthesis of such analogs required a method of preparation of phenoxathiin-4,6 or 1,9-dicarboxylic acid. The preparation of the 4,6-phenoxathiindicarboxylic acid (LXVIIa) by metalation seemed most promising and thus its synthesis via this route was investigated.

Gilman (24) showed that with the alkyllithiums the entering metal shows a strong tendency to replace a nuclear hydrogen atom alpha to a heteroatom. Thus, n-butyllithium (XLI) metalated thiophene in the 2-position (25), and dibenzothiophene (XLIX) in the 4-position to give the organometallics XLII and XLIII.



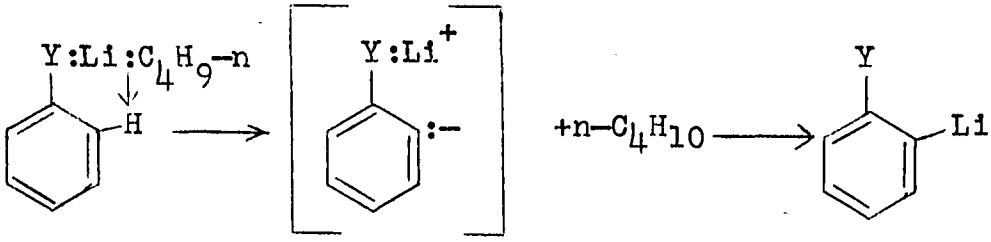
XLII



XLIII Li

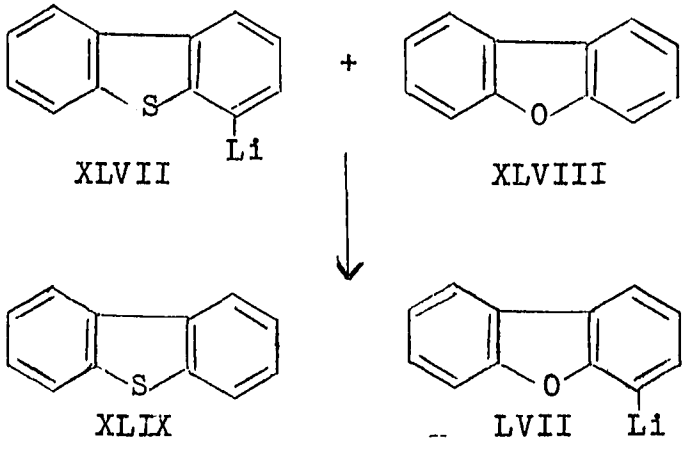
A simplified mechanism to rationalize the observed orientation was reported by Gilman (24). The organometallic ion-pair (XLIV) was pictured as forming a coordinate bond between the metal cation and a pair of electrons belonging to a heteroatom of the compound undergoing metalation. Subsequently or simultaneously, the carbanion portion of the organometallic ion-pair attacked an adjacent hydrogen atom, removing it as a proton. The coordination of the metal

cation with the hetero atom weakened the ionic bond between the metal and the carbanion and at the same time polarized the adjacent carbon–hydrogen bond facilitating the proton removal. The sequence is illustrated below by structures XLIV through XLVI.



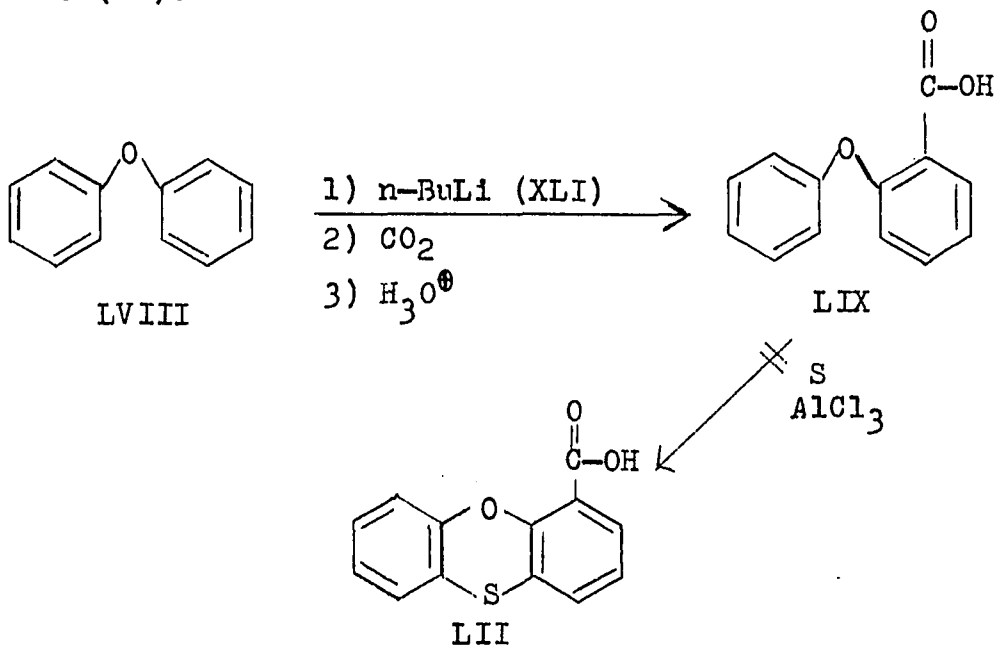
XLIV XLV XLVI

Gilman (36) showed that an ether linkage had a greater activating effect on the direction of metalation than did a sulfide linkage. Thus, when a mixture of dibenzofuran (XLVIII) and dibenzothiophene (XLIX) were treated with an insufficient quantity of n-butyllithium (XLI) only the dibenzofuran (XLVIII) underwent metalation. Moreover, 4-dibenzothienyllithium (XLVII) caused the metalation of dibenzofuran (XLVIII) in the 4-position to give dibenzothiophene (XLIX) and 4-dibenzofuryllithium (LVII).

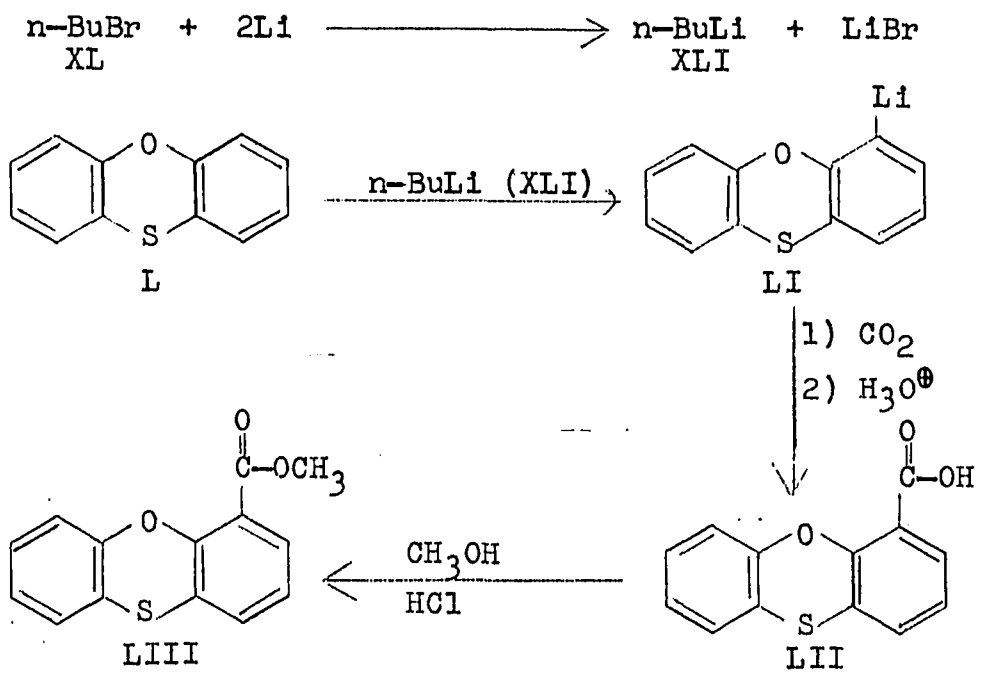


XLVII XLVIII XLIX LVII

This greater directing power of oxygen as compared with sulfur in organolithium metalation reactions was further confirmed by Gilman (36) by a study of the reaction of n-butyllithium (XLI) and phenoxathiin (L) which gave a 53% yield of the 4-substituted product, LII. Gilman (36) pointed out that this provided the only convenient method of preparation of the 4-substituted phenoxathiins starting with the parent phenoxathiin (L). Attempts to prepare 4-phenoxathiincarboxylic acid (LII) by ring closure experiments failed (36). For example, 2-carboxydiphenyl ether (LIX), prepared by the metalation of diphenyl ether (LVIII) with n-butyllithium (XLI), failed to give 4-phenoxathiincarboxylic acid (LII) on treatment with sulfur and aluminum chloride (26).



Repetition of Gilman's procedure (36) for the monometalation of phenoxathiin (L) using an ethereal solution of n-butyllithium (XLI), prepared from n-butylbromide (XL) and lithium, gave a 20.4% yield of pure 4-phenoxathiincarboxylic acid (LII). That the yield was inferior to that reported may have resulted from the difficulty in achieving the proper stoichiometry for the monometalation reaction. The use of a commercial n-butyllithium (XLI) solution in hexane* was more successful. The use of this n-butyllithium (XLI) led to the formation of 4-phenoxathiincarboxylic acid (LII) in average yields of 60% several times, and in one reaction the yield was 90%. The position of metalation was assumed from the identification of the carboxylation product as 4-phenoxathiincarboxylic acid (LII) by a comparison of physical constants of the acid and its methyl ester (LIII) with the literature values (36,38), and by the absorption spectra of LII and LIII.



*The author wishes to express appreciation to Dr. O. F. Beumel of the Foote Mineral Company for generous supplies of n-butyllithium.

Having found a satisfactory method of synthesis of 4-phenoxathiincarboxylic acid (LII), a route to the 4,6-phenoxathiindicarboxylic acid (LXVIIa) was sought. The formation of 34% of the 4,6-phenoxathiindicarboxylic acid (LXVIIa) was reported to occur from the reaction of phenoxathiin (L) with 2 equivalents of n-butyllithium (XLI) (41). The diacid was separated from 17.4% of 4-phenoxathiincarboxylic acid (LII) by extraction of the latter into benzene and from 8.9% of the 1,6-phenoxathiindicarboxylic acid (LXVIIIa) by fractional precipitation of the diacids from aqueous base. The impure 4,6-phenoxathiindicarboxylic acid (LXVIIa), m.p. 256-266^{c}, was precipitated by the weaker acid, acetic acid; while the impure 1,6-phenoxathiindicarboxylic acid (LXVIIIa), m.p. 295-316^{o}, required dilute hydrochloric acid. Repetition of Gilman's procedure (41) for the dimetalation of phenoxathiin (L) gave impure LXVIIa, m.p. 290-340^{o}, from acetic acid, and impure LXVIIa, m.p. 225-240^{o}, from dilute hydrochloric acid. Following recrystallization, LXVIIa, m.p. 270-273^{o}, was obtained in 3.47% yield; while LXVIIIa was obtained in 3.54% yield. The complex separation procedure may have led to considerable losses of the dicarboxylic acids. A different method of separation would be required, so new procedures for the separation of LXVIIa and

LXVIIIa in large quantities were investigated.

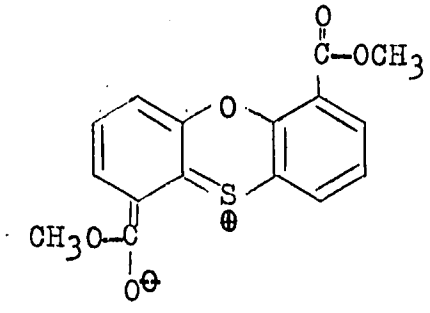
The greater reactivity of 4,6-phenoxathiindicarboxylic acid (LXVIIa) as compared with 1,6-phenoxathiindicarboxylic acid (LXVIIIa) with thionyl chloride suggested a separation based on the kinetics of acid chloride formation. The reaction of the dicarboxylic acid mixture with thionyl chloride was allowed to stand for 15 min. After filtration, the insoluble residue corresponded to LXVIIIa. Saponification of the acid chloride remaining after evaporation of the thionyl chloride gave 1.9% of LXVIIa, m.p. 270-273°, upon acidification.

The 4,6-phenoxathiindicarboxylic acid (LXVIIa) was found to be more soluble in diethyl aniline than the 1,6-dicarboxylic acid isomer. The attempts to use this difference for a separation gave 4.0% of LXVIIa, m.p. 250-269° and 9.0% of LXVIIIa, m.p. 345-350° (dec.).

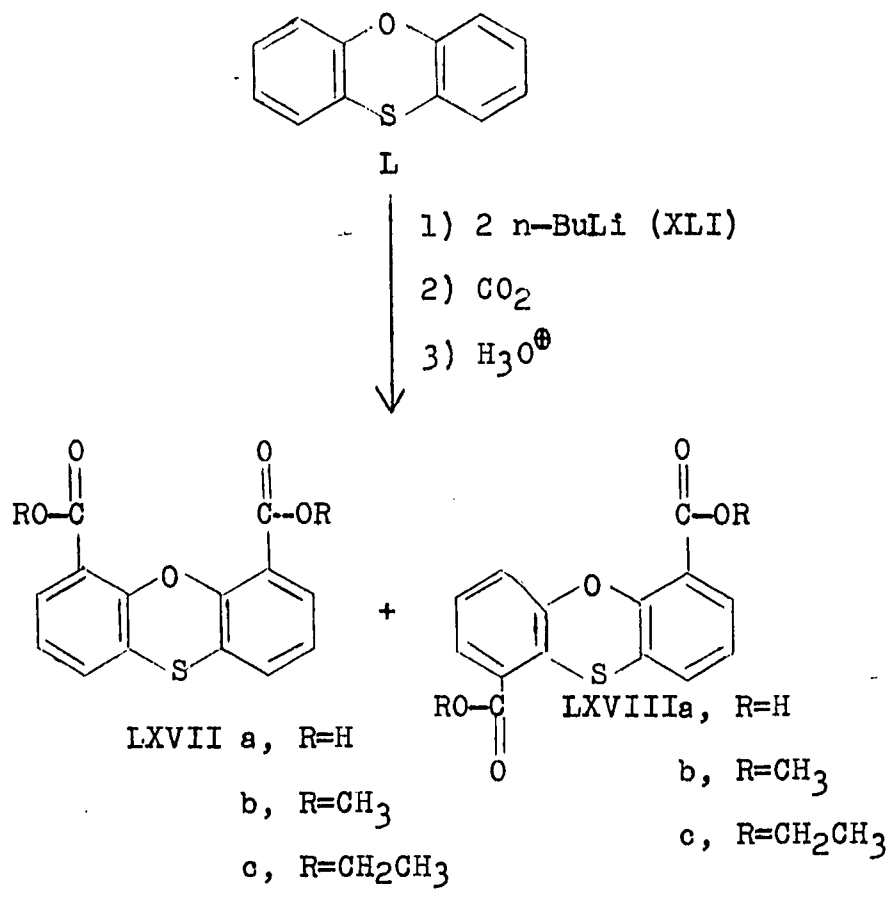
Other procedures were investigated in an attempt to increase the amount of dimetalation. The higher temperature obtained by changing the solvent from boiling ether to boiling hexane showed no change in product ratio. The use of 3 equivalents of n-butyllithium (XLI) per equivalent of phenoxathiin (L) gave yields of LXVIIc and LXVIIIc, isolated as the ethyl esters, of less than 1%.

The dicarboxylic acids, LXVIIa and LXVIIIa, obtained from these metalation reactions were identified as being the same as those obtained by Gilman (41) by the melting points of the acids and corresponding methyl esters, LXVIIb and LXVIIIb. The difference in the ultraviolet absorption spectra of dimethyl 4,6-phenoxathiindicarboxylate (LXVIIb)

also provided a method of distinguishing between these isomeric derivatives. The orbitals of the non-bonded electrons of oxygen and sulfur are presumed to overlap with the π-system of the aromatic rings, thereby increasing the electron density of the aromatic rings (28). In both esters, LXVIIb and LXVIIIb, the oxygen can exert an electron releasing effect by resonance with the carbonyl groups; but the sulfur can be in conjugation with the carbonyl group only in the 1,6-isomer (LXVIIIb). Sulfur is considerably less electronegative than oxygen, and thus greater overlap of its orbitals with the π-system of the aromatic rings is expected. Accordingly, if the excited state of LXVIIIb was stabilized by electron release from the sulfur atom, such as shown by structure LXIX, then a bathochromic and hyperchromic shift of the maximum absorption should be observed when the ultraviolet absorption spectrum of LXVIIIb was compared with that of LXVIIb. This hypothesis was confirmed by an observed 4 mμ bathochromic shift in the position of maximum absorption and a 1900 hyperchromic shift in its molecular extinction when the maximum absorption of LXVIIIb was compared to that of LXVIIb.



LXIX



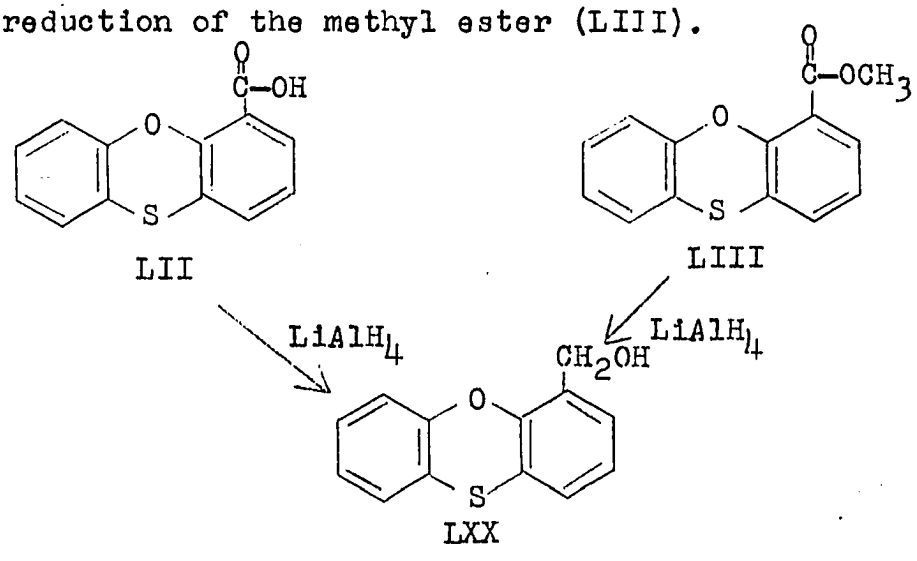
The failure of the direct metalation of phenoxathiin (L) to provide a satisfactory method of synthesis for 4,6-phenoxathiindicarboxylic acid (LXVIIa) required the consideration of another method of synthesis of LXVIIa. The reaction of 4-phenoxathiincarboxylic acid (LII) with n-butyllithium (XLI) would probably involve reaction of the organometallic with the carboxylic acid function rather than metalation of the ring. Conversion of the carboxylic acid

group of 4-phenoxathiincarboxylic acid (LII) to an electron donating group such as a carbinol would be expected to increase the susceptibility of the aromatic ring to metalation. Whether the electron releasing effect of the carbinol group would overcome the directing influence of the heterocyclic oxygen and lead to metalation in the 1-position could only be established by experimentation. If, however, the attack of n-butyllithium (XLI) was controlled by the heterocyclic oxygen atom, the resulting 4-hydroxymethyl-6-phenoxathiincarboxylic acid (LXXI) could be converted to the desired 4,6-phenoxathiindicarboxylic acid (LXVIIa) by oxidation.

To investigate this route to LXVIIa, several methods of conversion of 4-phenoxathiincarboxylic acid (LII) to the carbinol, 4-hydroxymethylphenoxathiin (LXX), which was also to be used as a model compound for oxidation, were considered. Three general methods are available for the conversion of a carboxylic acid to an alcohol (30). The first general method is the reduction of the ester by sodium and alcohol (32). The method is unsatisfactory with aromatic esters since complete reduction to Ar-CH_3 often occurs. The second general method is the catalytic hydrogenolysis of the ester over a copper chromite catalyst. However, this method is also complicated by partial reduction to Ar-CH_3. The third general method uses lithium aluminum hydride to effect reduction (39) of either the carboxylic acid or ester. This method appeared to be the most satisfactory since high yields of alcohols have been reported to result from the

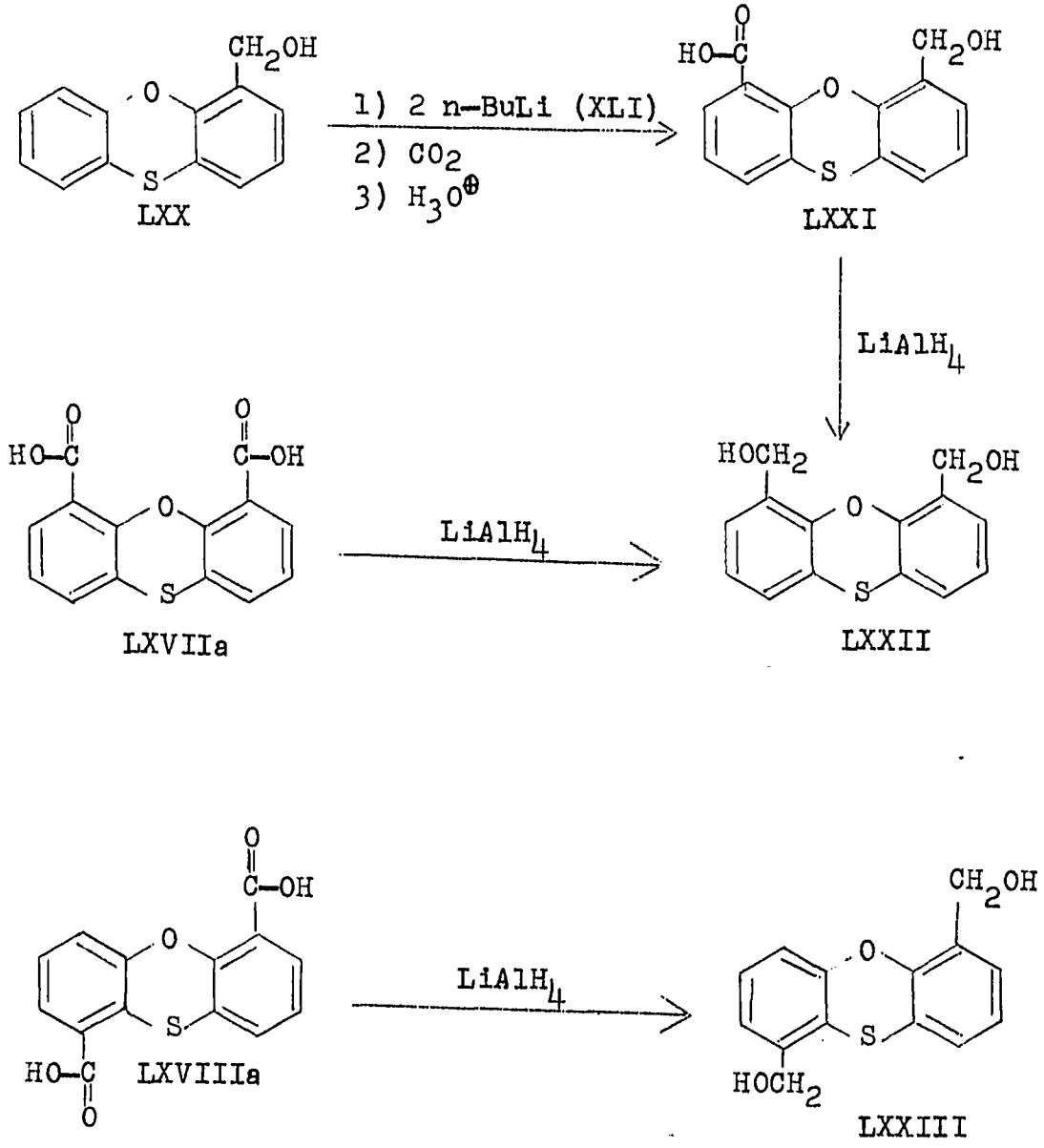
reduction of either ester or acid whether they are aliphatic or aromatic.

The conversion of 4-phenoxathiincarboxylic acid (LII) to 4-hydroxymethylphenoxathiin (LXX) was accomplished with lithium aluminum hydride by heating a tetrahydrofuran solution of LII under reflux. This reaction gave LXX in yields averaging 75%. These yields were considerably higher than the 6.6% yield of LXX obtained by the lithium aluminum hydride reduction of the methyl ester (LIII).



With this satisfactory method of synthesis of 4-hydroxymethylphenoxathiin (LXX) available, the metalation reaction was investigated. The metalation of 4-hydroxymethylphenoxathiin (LXX) with 2 equivalents of n-butyllithium (XLI), followed by carboxylation, led to the isolation of 4-hydroxymethyl-6-phenoxathiincarboxylic acid (LXX) in yields of approximately 55%. The structural assignment of the acid LXXI was established by reduction with lithium aluminum hydride to give the same dihydroxymethylphenoxathiin obtained by the lithium aluminum hydride reduction of 4,6-phenoxathiin-

dicarboxylic acid (LXVIIa). The fact that metalation took place on the 6-position and not on the 1-position was further substantiated by the fact that the reduction product LXXI was different from the reduction product, LXIII, of 1,6-phenoxathiindicarboxylic acid (LXVIIIa).



The nuclear magnetic resonance spectra of LXII and LXXIII can be rationalized if the structure LXXI is presumed for the metalation product of 4-hydroxymethylphenoxathiin (LXX). In general terms, the failure of a hydroxyl resonance to show coupling in nuclear magnetic resonance spectra determined in chloroform or carbon tetrachloride regardless of the presence of proton on an adjacent atom has been attributed to a rapid exchange of the proton from one hydroxyl to another. The exchange is sufficiently fast so that the lifetime of a proton attached to a specific oxygen is much less than the lifetime of 10^{-2}—10^{-3} sec. required for nuclear magnetic resonance to observe a single state (44). The result is that the proton of the hydroxyl group sees a number of environments which are averaged in the measurement to give a single peak rather than a split peak. In dimethyl sulfoxide, it has been proposed that hydrogen bonding of the alcohol to the solvent slows the rate of proton exchange sufficiently to permit detection of coupling (45,46).

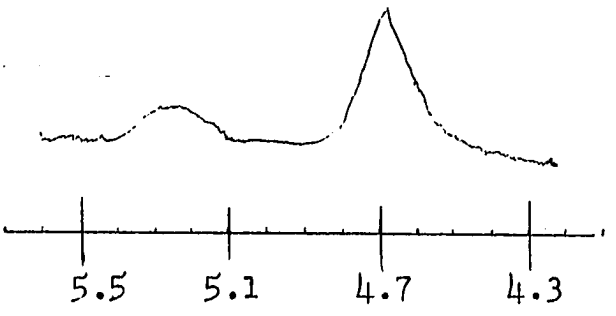
A comparison of the nuclear magnetic resonance spectra of 4,6-dihydroxymethylphenoxathiin (LXXII) and 1,6-dihydroxymethylphenoxathiin (LXXIII), determined in dimethylsulfoxide, indicated that the discussion above was consistent with the observed splitting in the latter case, but did not explain the absence of splitting in the former case. A possible explanation to account for these observed facts appeared to be in terms of hydrogen bonding. The infrared

spectrum of LXXII was determined as a solid mull rather than as a dilute solution; however, the absorption bands at 3460 $cm.^{-1}$ and 3375 $cm.^{-1}$ may indicate both intramolecular and intermolecular hydrogen bonding, respectively. The dimethyl sulfoxide would displace the intermolecular hydrogen bonding, but might not appreciably affect the intramolecular hydrogen bonding, if it were highly favored. If these conditions existed then this could account for the persistence of rapid hydroxyl proton exchange and the lack of splitting for the methylene and hydroxyl protons in LXXII. The 1,6-dihydroxymethylphenoxathiin (LXXIII), on the other hand, could not have any intramolecular hydrogen bonding between the hydroxyl hydrogens. The spectrum of LXXIII as a solid mull showed a broad band centered at 3250 $cm.^{-1}$ which may indicate only intermolecular hydrogen bonding. Presumably, the dimethyl sulfoxide slowed down the hydroxyl proton exchange sufficiently so that splitting was observed as expected. The broad hydroxyl peak showing fine splitting for 1,6-dihydroxymethylphenoxathiin (LXXIII) may well be due to overlapping of the two expected triplets. This interpretation may not be the only one to explain the observed spectra; however, it is consistent with the assigned structure, LXXI, of the metalation product of 4-hydroxymethylphenoxathiin (LXX).

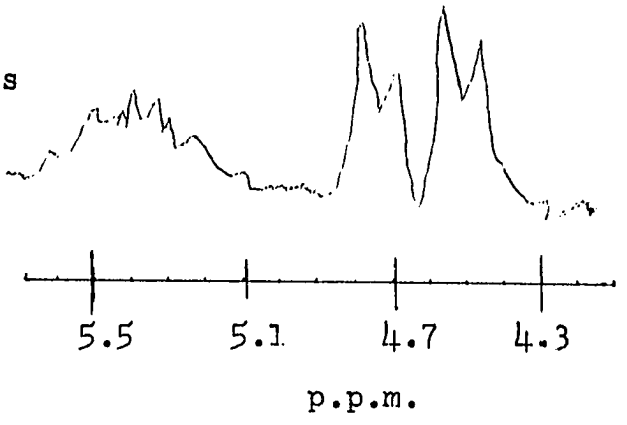
FIGURE II

Nuclear Magnetic Resonance Spectra of LXXII and LXXIII

Compound LXXII
Solvent DMSO
Filter Bandwidth 4 cps
R.F. Field 0.6 mG
Sweep Time 250 sec.
Sweep Width 500 cps
Sweep Offset 000 cps
Spectrum Amp. 40



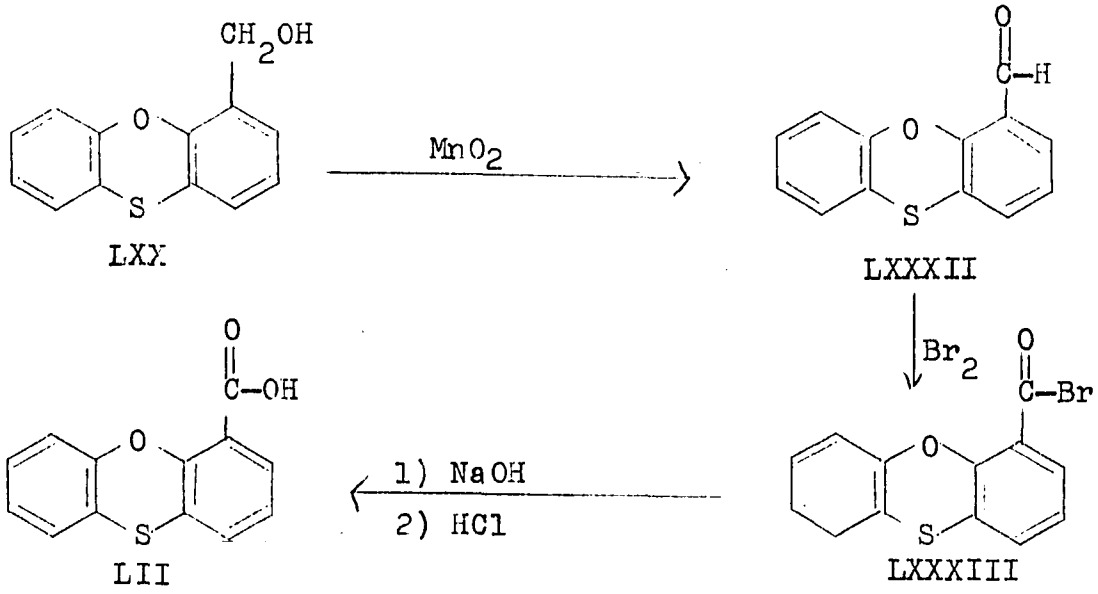
Compound LXXIII
Solvent DMSO
Filter Bandwidth-4 cps
R.F. Field 0.6 mG
Sweep Time 250 sec.
Sweep Width 500 cps
Sweep Offset 000 cps
Spectrum Amp. 40



The use of 4-hydroxymethyl-6-phenoxathiincarboxylic acid (LXXI) as an intermediate in the synthesis of 4,6-phenoxathiindicarboxylic acid (LXVIIa) required the development of a method of oxidation of the carbinol group to a carboxyl function. Since 4-hydroxymethylphenoxathiin (LXX) was more readily available, model experiments were performed on it to determine the effective methods of forming 4-phenoxathiincarboxylic acid (LII). These methods could then be applied to the oxidation of 4-hydroxymethyl-6-phenoxathiincarboxylic acid (LXXI) to get 4,6-phenoxathiindicarboxylic acid (LXVIIa).

Methods analogous to the oxidations of benzyl alcohol and benzaldehyde to benzoic acid were investigated for the oxidation of the aromatic carbinol function in LXX and LXXI to an aromatic carboxylic acid. The methods available are numerous (for leading references see references 63 and 64). The use of a number of these oxidative procedures is discussed below.

Manganese dioxide, prepared by the procedure of Stork (47), caused the oxidation of 4-hydroxymethylphenoxathiin (LXX) to 4-formylphenoxathiin (LXXXII) in good yields. LXXXII on reaction with bromine gave the acid bromide LXXXIII. This was hydrolyzed in fair yields to 4-phenoxathiincarboxylic acid (LII).



An attempt to use hypochlorite for the oxidation of 4–hydroxymethylphenoxathiin (LXX) and the reactions of silver oxide or oxygen for the oxidations of 4–formylphenoxathiin (LXXXII) to 4–phenoxathiincarboxylic acid (LII) failed to give the desired oxidation products. These reactions gave only recovered starting material.

As a result of the above oxidation study on 4–hydroxymethylphenoxathiin (LXX), manganese dioxide oxidation followed by bromine oxidation appeared to be a logical sequence for the conversion of 4–hydroxymethyl–6–phenoxathiincarboxylic acid (LXXI) to 4,6–phenoxathiindicarboxylic acid (LXVIIa). Unlike 4–hydroxymethylphenoxathiin (LXX), 4–hydroxymethyl–6–phenoxathiincarboxylic acid (LXXI) was not oxidized by manganese dioxide. The oxidation of 4–hydroxymethyl–6–phenoxathiincarboxylic acid (LXXI) was, therefore, attempted using bromine, chromic acid, oxygen or silver oxide oxidations; but the reactions either failed entirely or gave poor yields of the desired oxidation products.

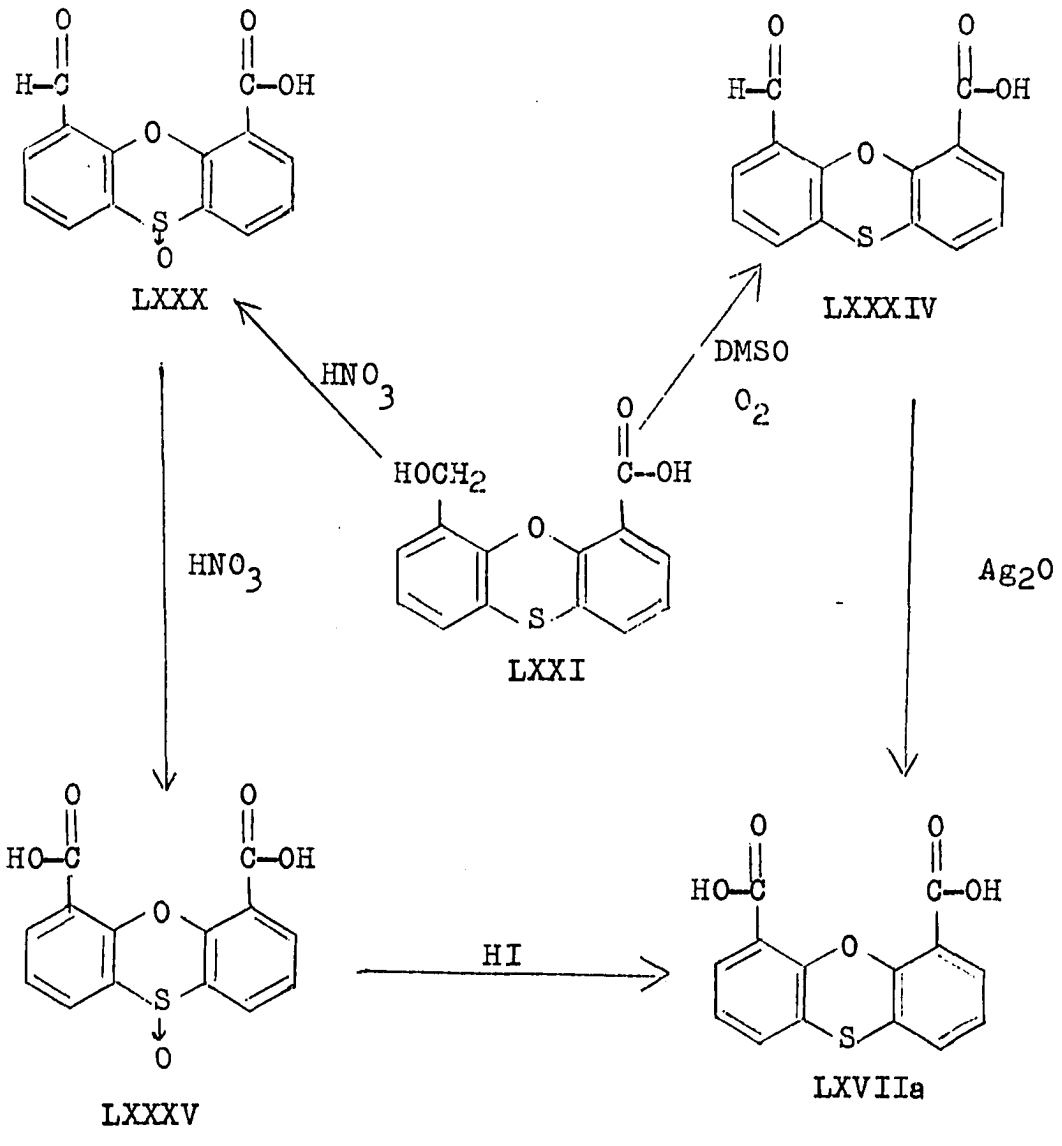
In the case of chromic acid oxidation, an infrared spectrum of the product mixture showed evidence of oxidation products containing a sulfoxide linkage. Due to these failures of oxidation of LXXI, other oxidative procedures were investigated.

Recently Traynelis (48) observed that the reaction of a solution of a benzyl alcohol and dimethyl sulfoxide with air led to good yields of the corresponding aldehyde. Contrary to the results of Searles (49) who found that dimethyl sulfoxide oxidation caused the conversion of aliphatic sulfides to sulfoxides, dimethyl sulfoxide oxidation of 4-hydroxymethyl-6-phenoxathiincarboxylic acid (LXXI) gave good yields of 4-formyl-6-phenoxathiincarboxylic acid (LXXXIV) with no trace of sulfoxide formation being observed. This apparent discrepancy in sulfide oxidation, however, can be readily understood when one considers that aromatic sulfides are less readily converted to sulfoxides than are aliphatic sulfides. The non-bonded electrons of the sulfur of the aromatic sulfide are delocalized due to resonance. The aldehyde, LXXXIV, on oxidation with silver oxide gave 4,6-phenoxathiindicarboxylic acid (LXVIIa) in good yields.

The reaction sequence for obtaining 4,6-phenoxathiindicarboxylic acid (LXVIIa), as discussed above, was operationally difficult. This was especially true at the dimethyl sulfoxide oxidation stage. The hazard of fire was constantly present, and the reaction required attention to

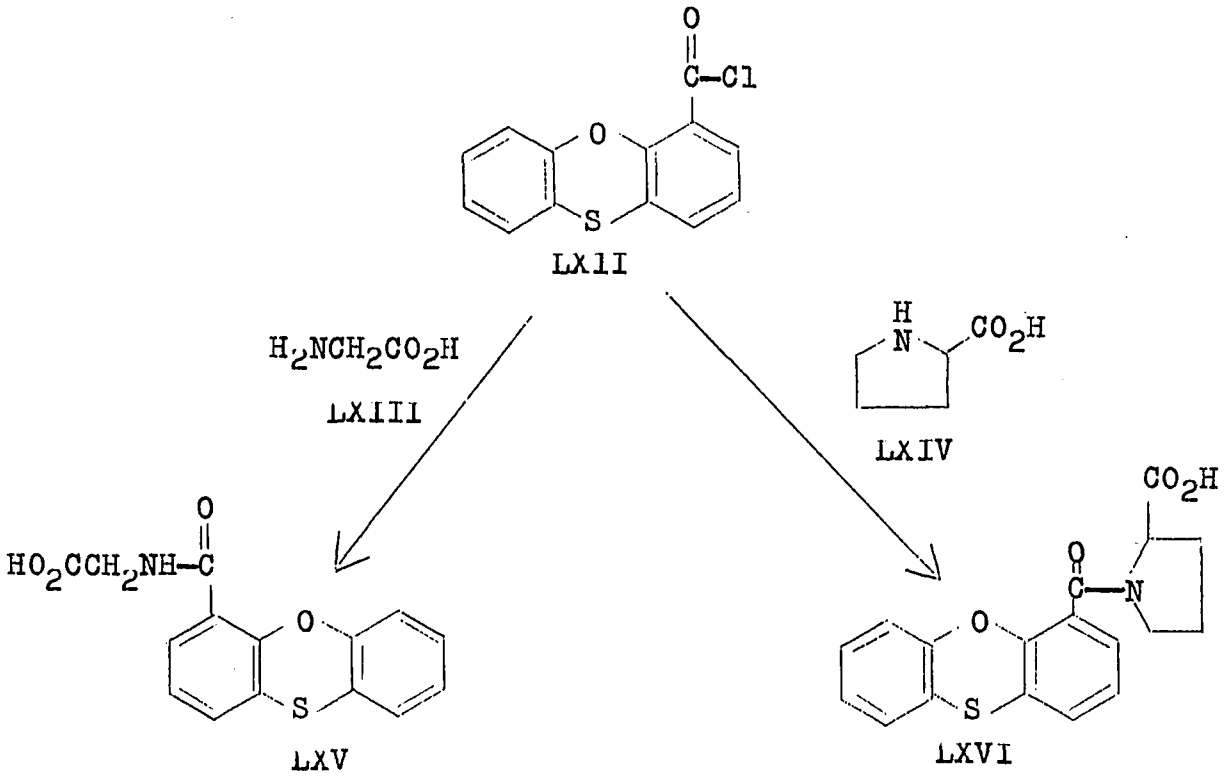
correct for evaporation and decomposition of the dimethyl sulfoxide. The decomposition products of dimethyl sulfoxide, such as methyl mercaptan and dimethyl sulfide, on heating at reflux had unpleasant odors and working with them was quite nauseating. Thus, instead of trying to avoid oxidation of the sulfide linkage to sulfoxide and/or sulfone during the oxidation of the carbinol, a procedure which would convert the carbinol and sulfide linkage to carboxylic acid and sulfoxide, respectively, was sought. The reduction of the sulfoxide could then be achieved by standard procedures to give LXVIIa.

Concentrated nitric acid has been used to oxidize carbinols to carboxylic acids and to oxidize sulfides to sulfoxides. As expected from the above combined studies, when 4-hydroxymethyl-6-phenoxathiincarboxylic acid (LXXI) was oxidized through the intermediate, LXXX, with concentrated nitric acid, excellent yields of 4,6-phenoxathiindicarboxylic acid-10-oxide (LXXXV) were obtained. The reduction of the sulfoxide linkage of LXXXV with hydriodic acid readily gave the desired 4,6-phenoxathiindicarboxylic acid (LXVIIa) in almost quantitative yields.



Having developed a satisfactory synthetic route to 4,6-phenoxathiindicarboxylic acid (LXVIIa), the major goal of the synthesis of a phenoxathiin analog of actinomycin was reached, for LXVIIa was a tricyclic, heterocyclic compound possessing carboxylic acid groups in the two peri-positions ortho to a hetero atom. However, the preparation of amide derivatives of LXVIIa was desired in order to obtain compounds even more closely analogous to actinomycin.

To investigate a route to these derivatives and to provide compounds for a comparison of any activity resulting from mono vs. disubstitution, Schotten–Baumann reactions were performed on the model compound, 4–phenoxathiincarboxylic acid chloride (LXII). The reaction of LXII with a number of amino acids under the usual Schotten–Baumann conditions employing a sodium hydroxide solution failed, however. That hydrolysis took place faster than reaction with a number of amino acids using this strong base was evident by the almost quantitative recovery of 4–phenoxathiincarboxylic acid (LII) on acidification of the reaction mixture. In only two cases was a reaction successful. These were the reactions with glycine (LXVIII) and with L(–)–proline (LXIV) to give N–(4–phenoxathiincarbonyl)glycine (LXV) and N–(4–phenoxathiincarbonyl)–L(–)–proline (LXVI), respectively. These products were obtained in small yields, and the reactions were of questionable reproducibility. Wu (22) had observed a similar difficulty in his studies on the reactions of 4–phenoxathiincarboxylic acid chloride (LXII) with amino acids. Elemental analysis and infrared spectra of the products LXV and LXVI were consistent with the structural assignments. The amide I and amide II bands for a secondary amide (40) were located at 1620 $cm.^{-1}$ and 1520 $cm.^{-1}$, respectively for LXV. Since there can not be an amide II band for a tertiary amide, only the amide I band was observed for LXVI at 1600 $cm.^{-1}$ with a shoulder at 1620 $cm.^{-1}$.



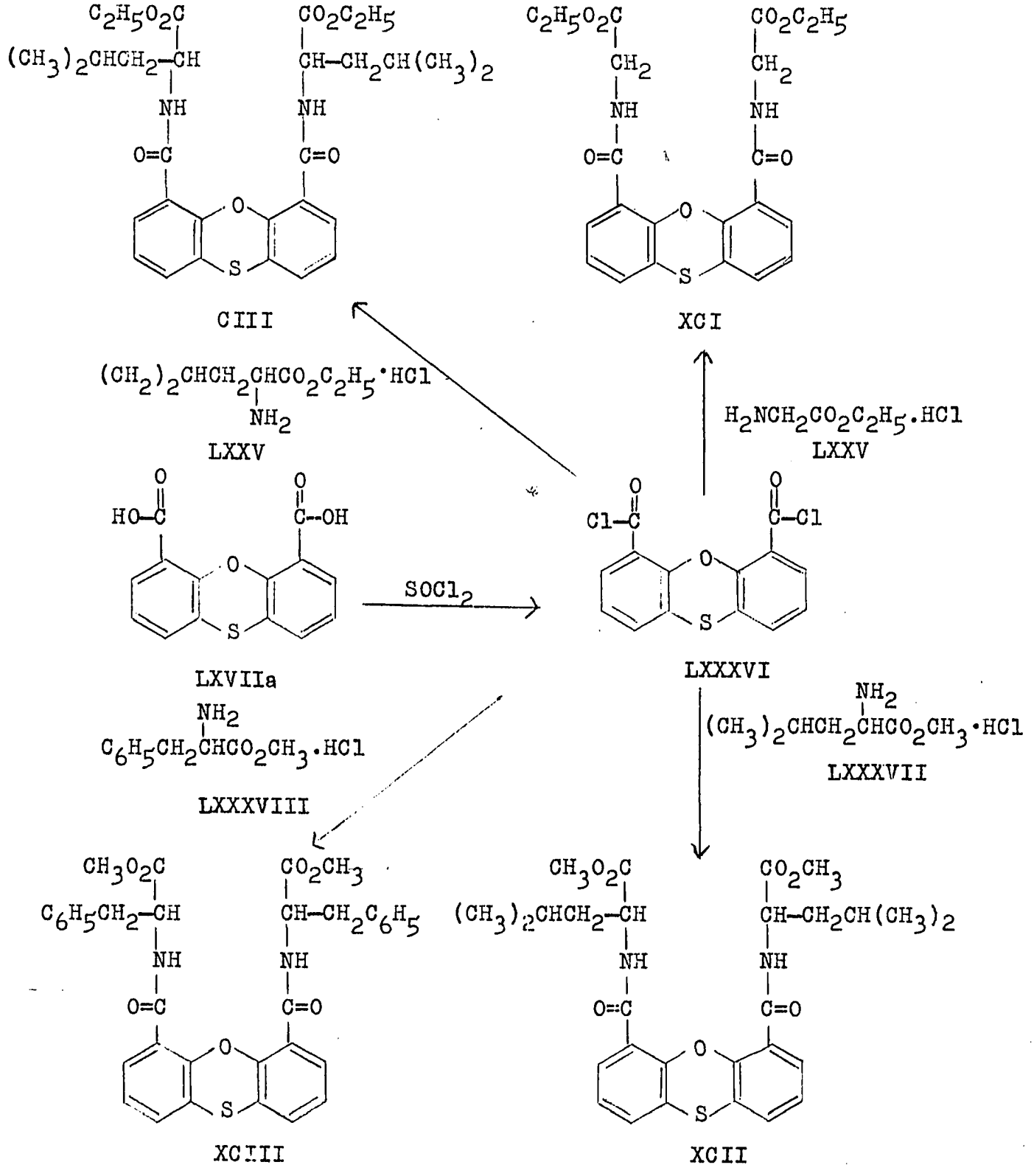
Having established the difficulty of reaction of 4-phenoxathiincarboxylic acid chloride (LXII) with amino acids in a sodium hydroxide solution, it was evident that modified Schotten-Baumann conditions should be used in the preparation of amide derivatives of 4,6-phenoxathiindicarboxylic acid (LXVIIa). The use of amino acid esters instead of amino acids would be advantageous because of the concommitant increase in nucleophilicity of the nitrogen atom. Furthermore, the hydrolysis of the acid chloride groups of LXXXVI could be virtually eliminated by dissolving the dicarboxylic acid chloride, LXXXVI, and the amino acid ester in benzene and stirring the resulting solution with an aqueous solution of the weak base, sodium bicarbonate.

When the acid chloride, LXXVI, was treated under these modified Schotten–Baumann conditions with glycine ethyl ester hydrochloride (LXXV), L(–)–leucine methyl ester hydrochloride (LXXXVII), L(–)–leucine ethyl ester hydrochloride (LXXXVIII) and L(–)–phenylalanine methyl ester hydrochloride (LXXXVIII) there were obtained good yields of diethyl N,N'–(4,6–phenoxathiinbiscarbonyl)diglycinate (XCI), dimethyl N,N'–(4,6–phenoxathiinbiscarbonyl)–L(–)–dilucinate (XCII), diethyl N,N'–(4,6–phenoxathiinbiscarbonyl)–L(–)–dilucinate monohydrate (CIII), and dimethyl N,N'–(4,6–phenoxathiinbiscarbonyl)–L(–)–diphenylalanate (XCIII). That reaction occurred on both acid chloride groups of LXXXVI was confirmed by elemental analysis, ultraviolet, infrared and nuclear magnetic resonance spectroscopy.

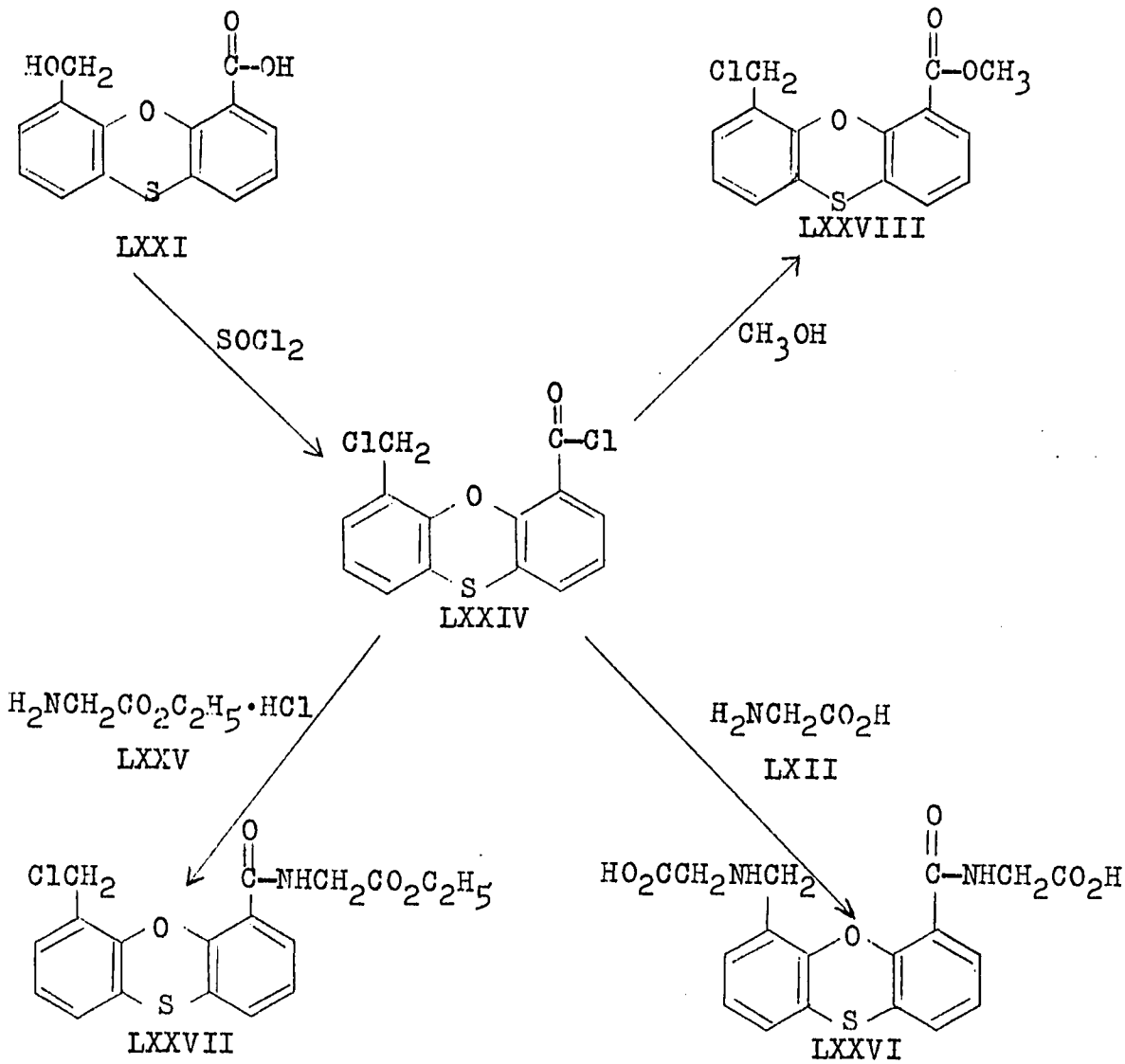
An alternate method was also investigated for the preparation of the amide derivatives of 4,6–phenoxathiindicarboxylic acid (LXVIIa). It was expected that the reaction of an amino acid or amino acid ester with 4–chloromethyl–6–phenoxathiincarboxylic acid chloride (LXXIV), prepared from 4–hydroxymethyl–6–phenoxathiincarboxylic acid (LXXI) and thionyl chloride, would take place with displacement of both halogen atoms. The resulting compound could then be oxidized by chromic acid (65) to give the same amide derivatives (LXI) that were prepared from the reaction of 4,6–phenoxathiincarboxylic acid chloride (LXXVI) and an amino acid or amino acid ester. Even if the reaction did not take place at the chloromethyl group, but only at the acid chloride group, derivatives would then have been available

to test any change in biological activity which would result on substitution of one of the peri-positions with a group that is not an amide derivative.

The reaction of 4-chloromethyl-6-phenoxathiincarboxylic acid chloride (LXXIV) with glycine (LXIII) and with glycine ethyl ester hydrochloride (LXXV) led to the formation of N,N'-(4-methyl-6-phenoxathiincarbonyl)diglycine (LXXVI) and ethyl N-(4-chloromethyl-6-phenoxathiincarbonyl) glycinate (LXXVII) respectively. The latter synthesis could also be accomplished, although in smaller yields, by heating a mixture of 4-chloromethyl-6-phenoxathiincarboxylic acid chloride (LXXV) and glycine ethyl ester hydrochloride (LXXV') in benzene. It is interesting to note that no reaction took place when LXXIV and LXXV were heated in triethyl amine. The wide melting point range (m.p. 140-147°) of the trace amount of product from the reaction with glycine indicated that the product may be impure. This was evident from the hydrogen analysis, which was 0.54% lower than that calculated for LXXVI. However, a negative Beilstein test and the infrared spectrum of this compound were consistent with the assigned structure, LXXVI. The assigned structure for LXXVII was confirmed by elemental analysis, a positive Beilstein test, and infrared and nuclear magnetic resonance spectroscopy.



The chloromethyl group appeared to be resistant to nucleophilic displacement by an amine, so further research leading to the preparation of amide derivatives (LXI) by this procedure was not pursued. The unusual formation of the chloromethyl group and its strong resistance to reaction was also indicated by the esterification of LXXIV with methyl alcohol to give, as the only product, methyl 4-chloromethyl-6-phenoxathiincarboxylate (LXXVIII).

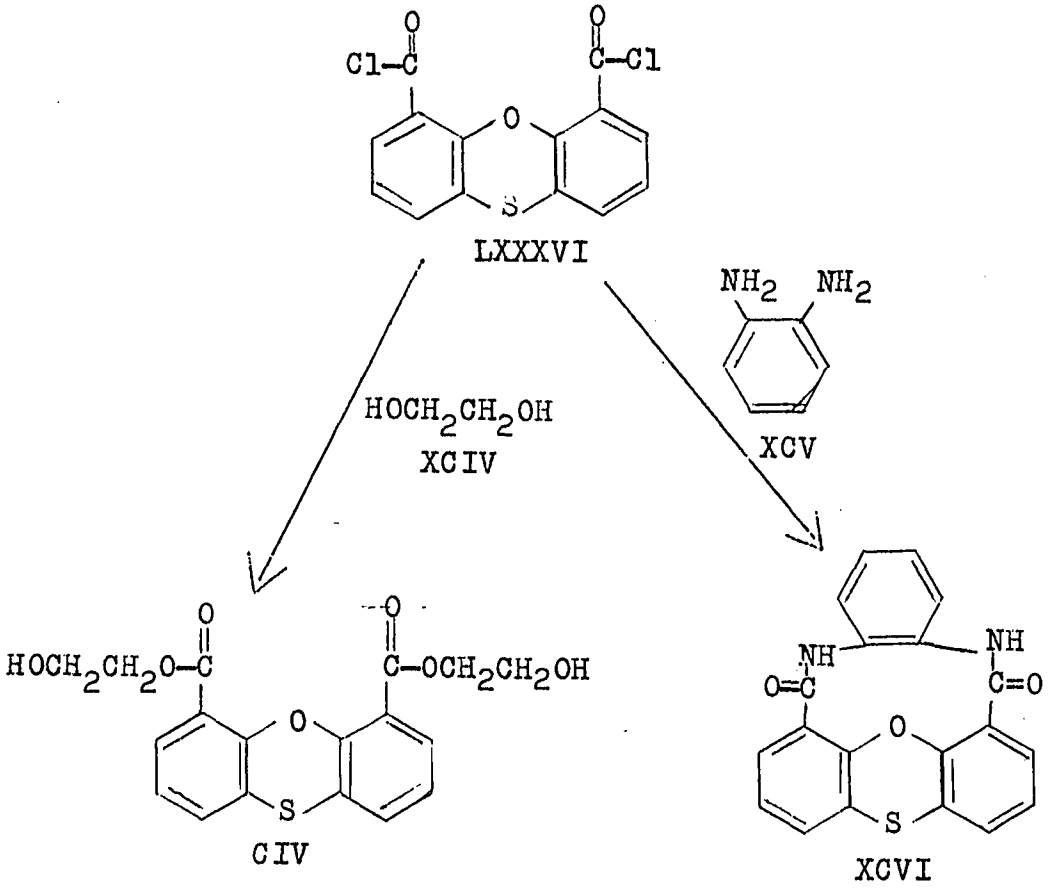


With the successful preparation of several amide derivatives of 4-phenoxathiincarboxylic acid (LII) and 4,6-phenoxathiindicarboxylic acid (LXVIIa) the goal of preparing phenoxathiin analogs of actinomycin was achieved. The successful synthetic procedures used to obtain this desired goal have thus led to derivatives of phenoxathiin (L) which are novel and to analogs of actinomycin which originate from a commercially available, biologically active tricyclic heterocyclic system.

Having accomplished the desired goal of preparation of several straight chain derivatives of phenoxathiin (L), it was of interest to attempt to join the 4- and 6-positions of phenoxathiin (L) through a cyclic structure. The preparation of such a phenoxathiin (L) derivative would be valuable in studying the conformation of the phenoxathiin ring system. It has been proposed that the tricyclic system may be capable of a butterfly type inversion; however, recent research on 1,4-dihydrobenzene would suggest that the ring system is planar. The important pharmacological activity of cyclic peptides also provided an interest in obtaining this novel type structure for screening.

The reaction of 4,6-phenoxathiindicarboxylic acid chloride (LXXXVI) with ethylene glycol (XCIV) failed to give a cyclic product, but presumably gave the acyclic product CIV. This was suggested by the presence of hydroxyl absorption at 3400 $cm.^{-1}$ and ester carbonyl absorption at 1720 $cm.^{-1}$ in the infrared spectrum of the product from this

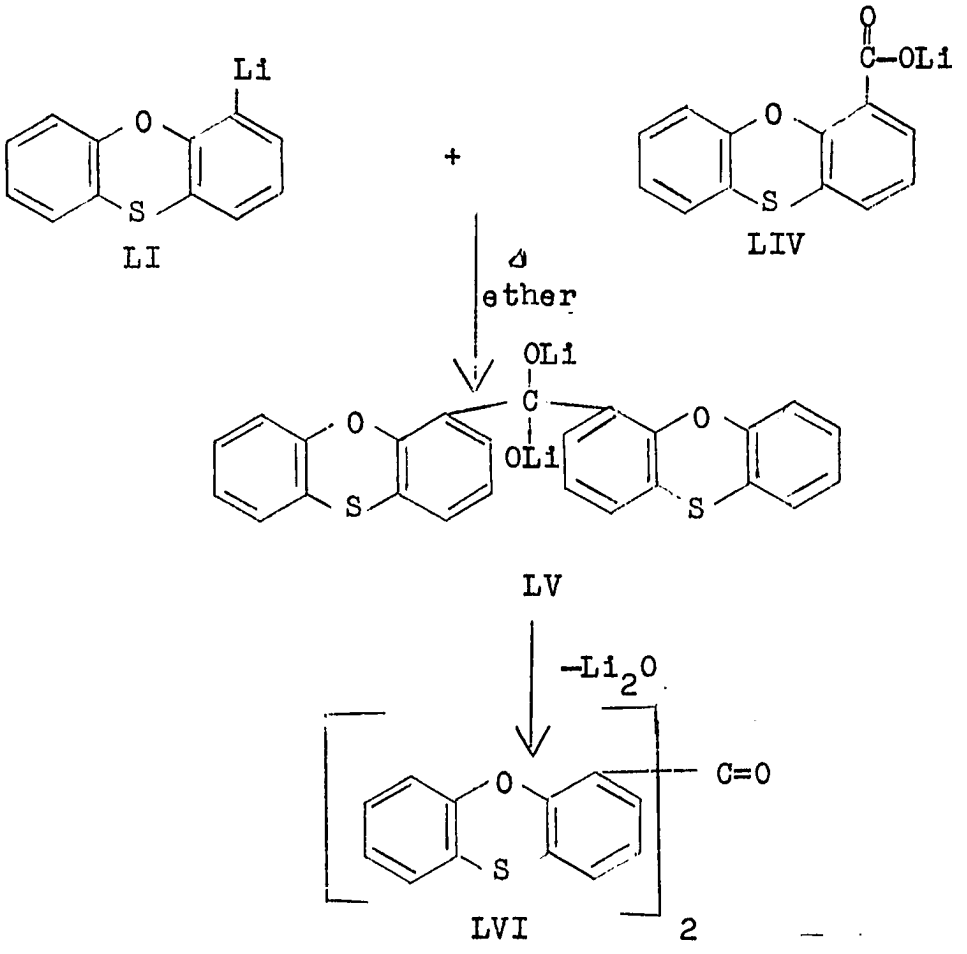
reaction. That the reaction of LXXVI with o-phenylenediamine (XCV), in which the amino groups are held rigidly by the benzene nucleus, probably gave the cyclic adduct XCVI was consistent with the elemental analysis of the product. The compound gave a negative Beilstein test and its infrared spectrum showed an amide I doublet at 1690 cm.$^{-1}$ and 1650 cm.$^{-1}$, and an amide II doublet at 1590 cm.$^{-1}$ and 1550 cm.$^{-1}$ for a secondary amide, thus, giving additional support to the assigned structure, XCVI, for the product of this reaction. Since the synthesis of such bridged compounds were not necessary to this thesis, these reactions were not investigated further.



An interesting side product was isolated from the monometalation reaction of phenoxathiin (L) with n-butyllithium (XLI). The structure of this side product was determined, and the method of its formation was investigated. The product showed maxima at 279 and 345 mμ in the ultraviolet spectrum, and the infrared spectrum showed a carbonyl stretching absorption at 1660 $cm.^{-1}$. These data strongly suggested a ketone carbonyl probably cross conjugated with two aromatic systems. The compound gave a positive 2,4-dinitrophenylhydrazine test with the formation of a red derivative, thus adding support to the assumption of the conjugated carbonyl. The elemental analysis suggested that the compound might be 4,4'-diphenoxathiinyl ketone (LVI). This structure would be in agreement with Gilman's observations (42,43) that ketones may result from metalation reactions under certain conditions. The successful synthesis of 4,4'-diphenoxathiinyl ketone (LVI) from the reaction of 4-phenoxathiinyl lithium (LI) and lithium 4-phenoxathiincarboxylate (LIV) in 6.4% yield confirmed the structural assignment of this second product obtained in a 1.7% yield from the monometalation reaction.

Although water was used in the work up of the monometalation reaction products, precautions were taken to avoid contact of water with the reaction mixture at all times in the direct synthesis of LVI. After heating an anhydrous ether-hexane solution of LI and LIV under nitrogen, the reaction mixture was filtered and the solvent was allowed to

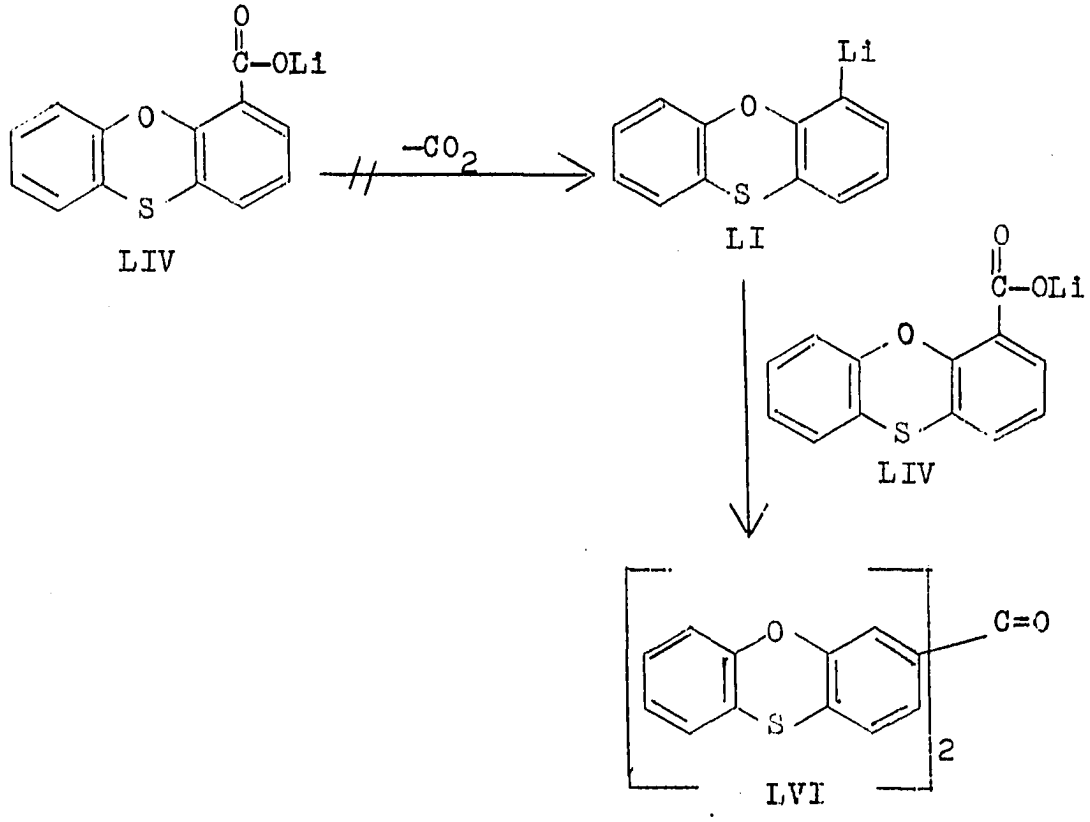
evaporate from the filtrate to give LVI. It appears unlikely that LVI was soluble in ether and that atmospheric moisture caused the hydrolysis of LV to LVI. Therefore, the formation of LVI in the absence of water indicated that the direct elimination of lithium oxide may have taken place, although in small yields, contrary to the statement of Gilman (42,43) that hydrolysis was necessary to free the carbonyl compound.



The assigned structure of the ketone (LVI) was further complemented by the nuclear magnetic resonance spectrum which showed a quartet at 5.75 p.p.m. (2 hydrogens), an aromatic complex at 6.97 p.p.m. (12 hydrogens) and a quartet at 7.47 p.p.m. (2 hydrogens). It is a well known fact that a carbonyl group attached to a benzene ring may have a strong deshielding effect on the ortho-hydrogens (65). The observed quartet at low field indicated that this was as expected for the 3 and 3'-positions. The paramagnetic shift noted for the higher field quartet may be explained by a shielding effect on the protons of the 6 and 6'-positions by the π system of the opposing aromatic moiety.

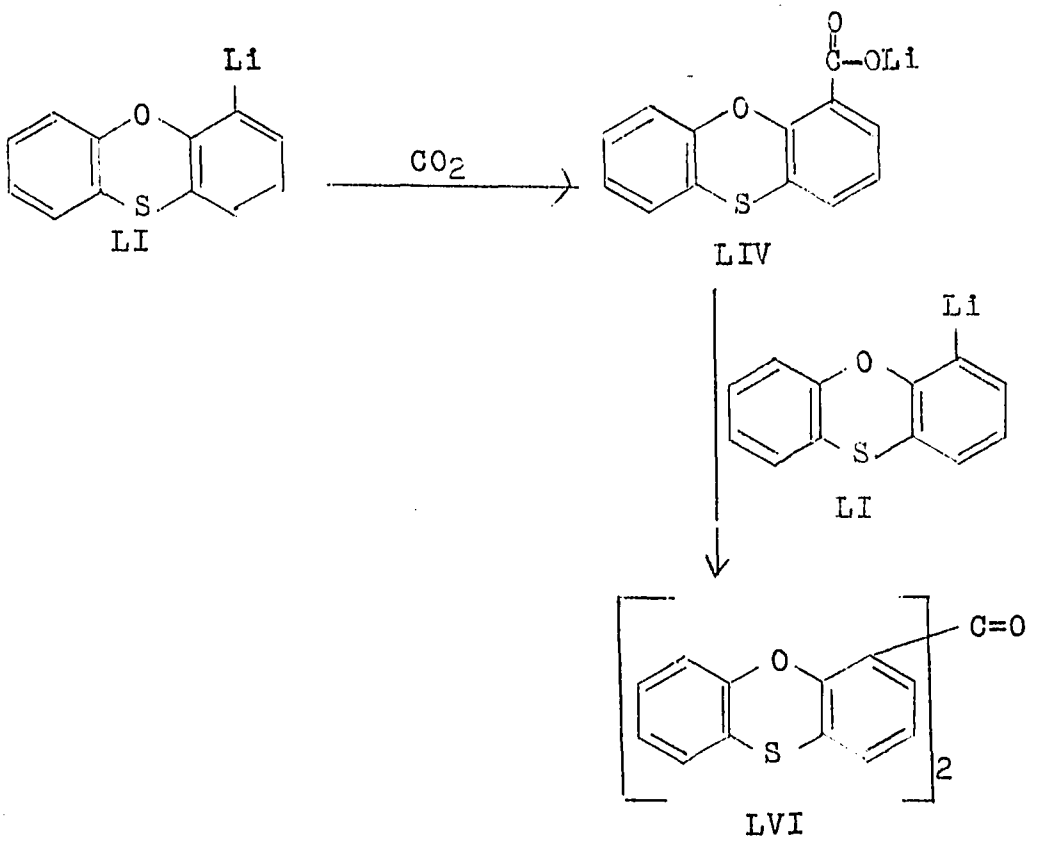
Steric factors may be mentioned as an explanation for the low yield (1.7%) of ketone (LVI), obtained in the monometalation reaction. This explanation is correlated with the fact that p-tolyllithium on carboxylation at 0-15° gave 1% of p-toluic acid and 77-81% of di-P-tolyl ketone; whereas, o-tolyllithium on carboxylation at the same temperature gave o-toluic acid in 35% yield as the only product (42). Other factors which may account for the low yield of ketone from the metalation reaction are: (1) solid carbon dioxide was used to provide a large local concentration of carbon dioxide for the carboxylation step, and (2) a low temperature was used for the carboxylation step to decrease the formation of gaseous carbon dioxide.

Having established the structure of the ketone from the monometalation reaction of phenoxathiin (L), it was desirable to examine its mode of formation. Three possible synthetic routes were investigated. (1) Lithium 4-phenoxathiincarboxylate (LIV) may have undergone decarboxylation in part to 4-phenoxathiinyllithium (LI) which then underwent reaction with excess lithium 4-phenoxathiincarboxylate (LIV) to form the ketone, LVI. That such a decarboxylation step did not occur was verified by the lack of formation of phenoxathiin (L), which would have resulted from the reaction of excess LI with water during the aqueous work-up, or ketone (LVI) when a mixture of lithium 4-phenoxathiincarboxylate (LIV) and tetrahydrofuran was heated under reflux.



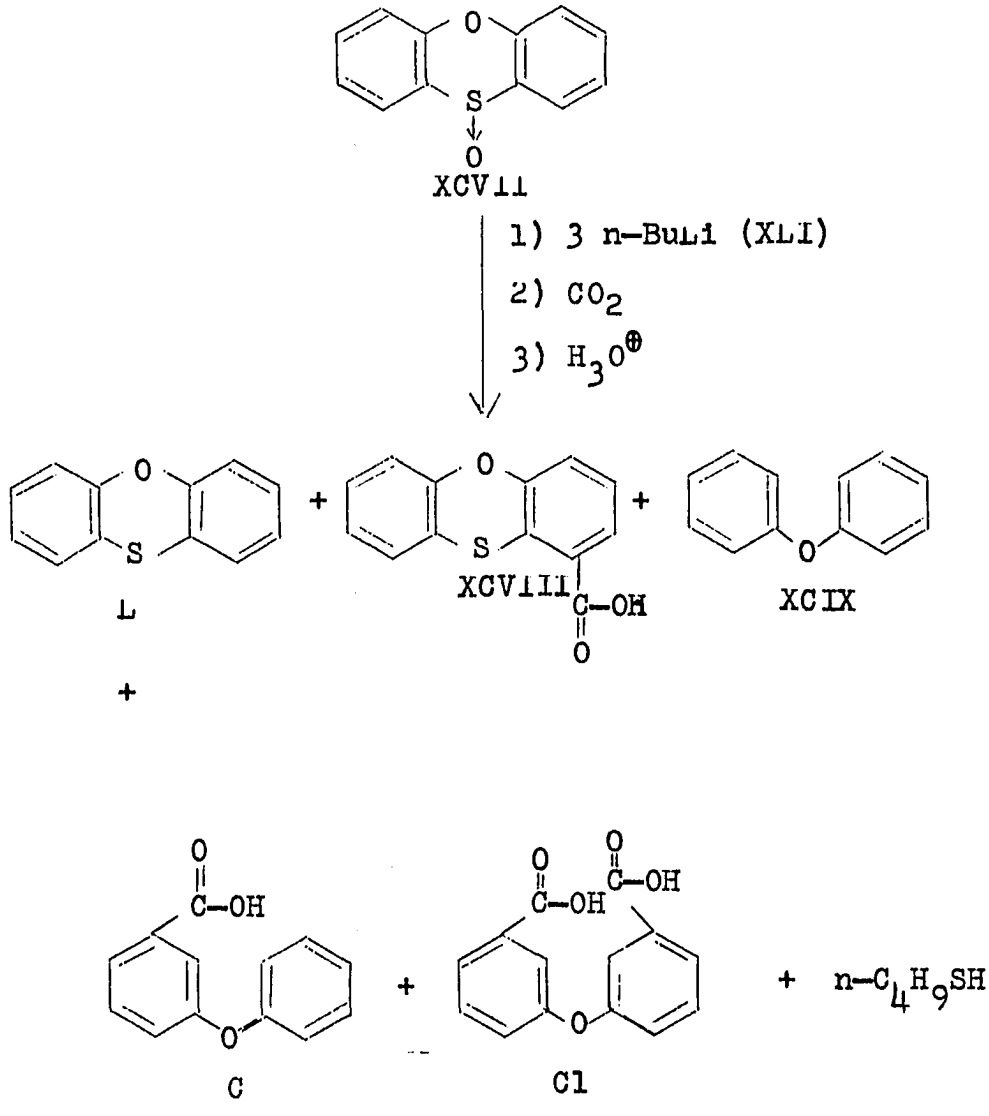
(2) 4-Phenoxathiinyllithium (LI) may have undergone reaction with carbon dioxide from the atmosphere to give lithium 4-phenoxathiincarboxylate (LIV) which gave addition with excess 4-phenoxathiinyllithium (LI) to form the ketone (LVI). This possibility was supported by the observation of Gilman (42) that phenyllithium was converted to benzophenone by the presence of small volumes of gaseous carbon dioxide. That this reaction did not occur was verified by the lack of formation of ketone LVI when the metalation reaction was decomposed by being poured into water rather than on solid carbon dioxide.

(3) 4-Phenoxathiinyllithium (LI) may have undergone reaction with a limited amount of carbon dioxide gas from the sublimation of solid carbon dioxide to give lithium 4-phenoxathiincarboxylate (LIV) which reacted with excess 4-phenoxathiinyllithium (LI) to form the ketone (LVI). That this was the case was verified by carboxylation of the metalation reaction with carbon dioxide gas instead of solid carbon dioxide to form the ketone LVI in yields of less than 2%.

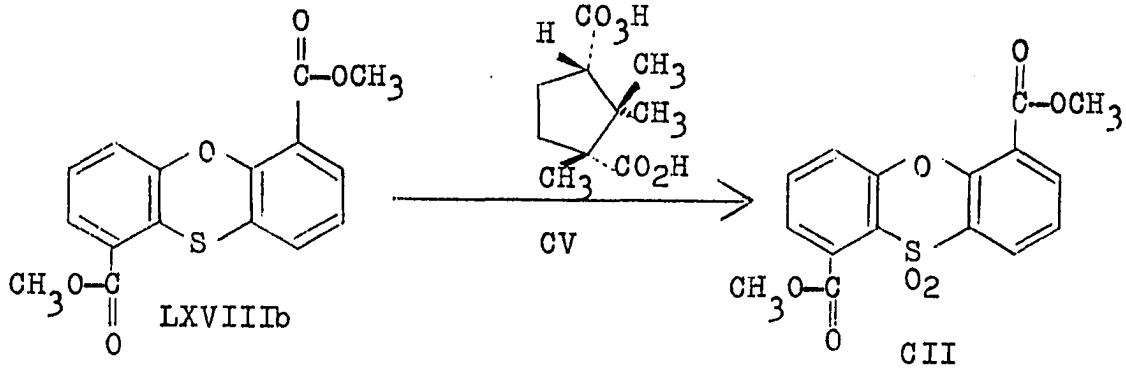


It was of interest to investigate briefly the possibility of synthesis of a phenoxathiin analog of actinomycin in which the carbonyl function of the heteroaroyl moiety was located at a position ortho to the sulfur heteroatom and not the oxygen heteroatom as was previously discussed. Gilman (53) and Shirley (54) reported that the reaction of phenoxathiin-10-oxide (XCVII) with 3 equivalents of n-butyllithium (XLI) led to a mixture of phenoxathiin (L), 1-phenoxathiin-carboxylic acid (XCVIII), diphenyl ether (XCIX), 2-carboxydiphenyl ether (C), 2,2'-dicarboxydiphenyl ether (C), and n-butyl mercaptan. When the reaction of phenoxathiin-10-oxide (XCVII), prepared by the procedures of Tomita (51) and Gilman (52), and 3 equivalents of n-butyllithium (XLI) was repeated in this laboratory, the only products isolated were

CI, valeric acid and recovered XCVII. The attempted synthesis of 1-phenoxathiincarboxylic acid (XCVIII), which had been pursued only as a side interest, was not investigated further.



It was desirable to investigate whether or not asymmetry could be introduced in a planar and diaryl system by an asymmetric oxidizing agent. An asymmetric oxidation by D(+)-percamphoric acid (CV) would be expected to lead to an optically active compound starting with LXVIIIa, LXVIIIb, or LXVIIIc. Since no attempts at asymmetric oxidations of diaryl sulfides have been reported, the results of this brief investigation would be novel. The attempted asymmetric oxidation on the diaryl sulfide, dimethyl 1,6-phenoxathiincarboxylate (LXVIIIb) led, however, to the formation of dimethyl 1,6-phenoxathiindicarboxylate-10-dioxide (CII) using D(+)-percamphoric acid (CV), prepared by the procedure of Milas (59). Therefore, this study was abandoned.



EXPERIMENTAL

Melting points were determined on a Köfler hot stage apparatus and are uncorrected.

Infrared absorption spectra were determined on a Perkin-Elmer Model 137-B Infracord Spectrophotometer, or on a Perkin-Elmer Model 337 Infracord Spectrophotometer equipped with sodium chloride optics, and were reported in $cm.^{-1}$. Each infrared spectrum is assigned a spectrum number which refers to a certain infrared spectrum on a particular infrared spectrophotometer at the University of New Hampshire. Infrared spectra of the liquid samples were determined as films, and solid materials were determined as mulls in Halocarbon oil (from 4000 $cm.^{-1}$ to 1300 $cm.^{-1}$) and in Nujol (from 1300 $cm.^{-1}$ to 600 $cm.^{-1}$). The bands are indicated as strong(s), medium(m), weak(w), or shoulder(sh).

Ultraviolet spectra were determined using a Perkin-Elmer Model 4000 Spectracord Recording Spectrophotometer, and were reported in mu.

Elemental analyses on compounds indicated by an asterisk were determined by Schwarzkopf Microanalytical Laboratories, Woodside, New York. Elemental analysis on the remainder of the compounds were determined with a F&M Carbon, Hydrogen, Nitrogen Analyzer, Model 180.

Molecular weights were determined on a Mechrolab Vapor Pressure Osmometer, Model 301A.

Preparation of n-Butyllithium(LXI). Into a 1-l. 3-necked flask equipped with a Hirshberg stirrer, dropping funnel, and thermometer were placed 400 ml. of anhydrous ether. After sweeping the apparatus with nitrogen, 20.0 g. (2.88 g. atoms) of lithium wire, cut approximately 5 mm. in length, were washed with ether and placed in the reaction flask. Nitrogen was passed through the system from this point to the conclusion of the preparation. With the stirrer started, about 60 drops of a solution of 104.0 g. (0.76 mole) of n-butyl bromide(XL) in 200 ml. of anhydrous ether were added from the dropping funnel to initiate the reaction. The start of the reaction was evidenced by a cloudiness of the reaction mixture. The reaction mixture was then cooled to -10° with an acetone-solid carbon dioxide bath and the remainder of the n-butyl bromide(XL) and ether solution was added during a 0.5 hr. period. After the addition was completed, the reaction mixture was allowed to warm to 10°. The reaction mixture was then filtered through glass-wool, and the filtrate was diluted to 800 ml. with anhydrous ether. The yield was estimated (37) at 80-90%.

The n-butyllithium(XLI) solutions, as prepared above, were used immediately.

However, most of the metalation reactions were carried out using n-butyllithium(XLI) provided by Foote Mineral Company.

Preparation of 4-Phenoxathiincarboxylic Acid(LII).

Procedure A - To 300 ml. of an ethereal solution of n-butyllithium(XLI) prepared from 208.0 g. (1.52 mole) of n-butyl bromide(XL) and 40.0 g. (5.76 g. atoms) of lithium, was added 80.0 g. (0.40 mole) of phenoxathiin(L) and the reaction was stirred for 51 hr. under nitrogen at room temperature with a Hirshberg stirrer. The solution was then poured, under nitrogen, onto a slurry of solid carbon dioxide in ether. The mixture was allowed to stand until all the solid carbon dioxide had sublimed, and 200 ml. of water were added to the reaction mixture. The aqueous phase was separated and acidified with 5% hydrochloric acid to cause the precipitation of 35.5 g. of highly impure 4-phenoxathiincarboxylic acid(LII), m.p. 150-280°. The impure compound was stirred with 40% sodium hydroxide and the mixture was allowed to stand for 5 hr. Filtration through a sintered glass funnel and acidification of the filtrate gave a product of m.p. 162-164°. Three recrystallizations of the crude solid from glacial acetic acid gave 20.0 g. (20.4%) of pure 4-phenoxathiincarboxylic acid(LII), m.p. 171-173°; Lit. (36), m.p. 170-172°.

Procedure B – To a solution of 40.0 g. (0.20 mole) of phenoxathiin(L) in 200 ml. of anhydrous ether was added 85.2 g. (0.20 mole) of 15.03% n-butyllithium(XLI) in hexane solution, and the resultant solution was stirred for 40 hr. at room temperature with a magnetic stirrer in a sealed system under nitrogen. The solution was then poured, under nitrogen, onto a slurry of solid carbon dioxide in ether. After all the solid carbon dioxide and solvent had evaporated, the residue was washed with water and the slurry was filtered to separate the insoluble starting phenoxathiin(L) from the lithium carboxylate salt(LIV). Acidification of the filtrate caused the precipitation of 29.0 g. (59.3%) of impure 4-phenoxathiincarboxylic acid(LII), m.p. 162–172°. Two recrystallizations of the solid from glacial acetic acid gave 13.5 g. (27.6%) of pure 4-phenoxathiincarboxylic acid(LII), m.p. 173–175°.

Because of the need for large amounts of 4-phenoxathiincarboxylic acid(LII), this procedure was repeated many times. The highest yield of impure 4-phenoxathiincarboxylic acid(LII) obtained was 91.2% of melting point 160–168°; however, the average yield was approximately 70%. Recrystallization of the crude material from glacial acetic acid decreased the average yield to approximately 50% of pure compound, m.p. 171–173°. The average yield of pure compound could be increased to approximately 60% by collecting a second crop of 4-phenoxathiincarboxylic acid(LII) on evaporation of the solvent under aspirator pressure.

A time study designed to arrive at the highest yield of product indicated that the reaction could be carried out in 20 hr. instead of 40 hr. without any substantial loss in yield.

Anal. Calcd. for $C_{13}H_8O_3S$: C, 63.92; H, 3.30. Found: C, 64.05; H, 3.05.

Infrared Spectrum (Model 137-B, No. 5): 3000(m), 1680(s), 1600(m), 1470(m), 1420(s), 1275(m), 1220(m), 1160(w), 1130(w), 1080(w), 1030(w), 940(m, broad), 890(m), 850(m, broad), 810(m), 770(sh), 755(s), 730(m), 720(m), 675(w).

Preparation of Methyl 4-Phenoxathiincarboxylate(LII). Procedure A - A solution of 2.0 g. (8.1 mmoles) of 4-phenoxathiincarboxylic acid(LII) in 50 ml. of absolute methyl alcohol was saturated with hydrogen chloride and the resulting solution was heated under reflux for 3 hr. The solution was then poured into 150 ml. of ice water to cause the precipitation of a dark brown oil, which on distillation under reduced pressure gave 1.50 g. (71.8%) of methyl 4-phenoxathiincarboxylate(LIII), b.p. 160-185° at 0.9 mm; Lit. (38) b.p. 183-187° at 1 mm.

Procedure B - To 20.0 g. (0.081 mole) of 4-phenoxathiincarboxylic acid(LII) was added 25 ml. of thionyl chloride, and the solution was heated under reflux for 30 min. After removal of excess thionyl chloride by evaporation under reduced pressure, 30 ml. of methyl alcohol was added to the impure 4-phenoxathiincarboxylic acid chloride

(LXII) residue. The solution was refluxed for 10 min., and poured into 100 ml. of water. The resultant oil was taken up with ether and the ether separated and removed by flash-distillation under reduced pressure. The oil which was left behind was distilled under reduced pressure to give 12.0 g. (73.5%) of methyl 4-phenoxathiincarboxylate(LIII), b.p. 196-198° at 2.7 mm; Lit. (38) b.p. 183-187° at 1 mm.

Infrared Spectrum (Model 137-B, No. 1575): 3450(m, broad), 1720(s), 1600(m), 1470(w), 1420(s), 1275(w), 1220(w), 1200(w), 1160(m), 1130(m), 1080(m), 1030(s), 960(m), 940(w), 875(m), 860(w), 755(s, broad), 730(sh), 720(sh), 675(m).

Reaction of Phenoxathiin(L) with One Equivalent of n-Butyllithium(XLI). To a solution of 100.0 g. (0.50 mole) of phenoxathiin(L) in 200 ml. of ether was added 212.8 g. (0.50 mole) of a 15.05% solution of n-butyllithium(LXI) in hexane, and the solution was stirred in a sealed atmosphere of nitrogen for 30 hr. The solution was then poured on a slurry of solid carbon dioxide in ether, and the reaction was allowed to stand until all the solid carbon dioxide and solvent had evaporated. Water was added to the resulting residue, and the heterogeneous mixture was resolved by filtration.

The 12.5 g. of insoluble residue from the filtration was recrystallized twice from acetone to give 3.5 g. (1.7%) of 4,4'-diphenoxathiinyl ketone(LVI), m.p. 159-164°. Superimposability of the infrared spectrum of 4,4'-diphenoxathiinyl ketone(LVI) with that of an authentic sample prepared from the reaction of 4-phenoxathiinyllithium(LI) and lithium 4-

phenoxathiincarboxylate(LIV) and the mixture melting point of the two solids showed that the compounds were identical.

Evaporation of the acetone gave a sticky solid which after trituration with cold petroleum ether and recrystallization from methyl alcohol gave 2.5 g. of recovered phenoxathiin(L), m.p. 54–56°. Superimposability of the infrared spectrum of the recovered phenoxathiin(L) with that of the starting phenoxathiin(L) and the mixture melting point of the two solids showed that the compounds were identical.

The original aqueous filtrate was extracted with 200 ml. of ether, and evaporation of the ether from the extract gave only a trace of impure 4–phenoxathiincarboxylic acid(LII). A 5% solution of hydrochloric acid was added to the aqueous phase causing the precipitation of 82.5 g. of impure 4–phenoxathiincarboxylic acid(LII), m.p. 145–160°. Recrystallization from glacial acetic acid gave in two crops 68.1 g. (56.0%) of 4–phenoxathiincarboxylic acid(LII), m.p. 167–171°.

In order to determine whether the 4,4'–diphenoxathiinyl ketone(LVI) could have been formed by a reaction of 4–phenoxathiinyllithium(LI) with carbon dioxide present in the air, the following experiment was run. To a solution of 10.0 g. (0.05 mole) of phenoxathiin(L) in 100 ml. of anhydrous ether was added 21.3 g. (0.05 mole) of a 15.05% n–butyllithium(XLI) in hexane solution, and the resultant solution stirred for 24 hr. at room temperature with a magnetic stirrer in a sealed system under nitrogen.

The solution was then slowly poured into 200 ml. of water. A quantitative recovery of phenoxathiin(L), m.p. 54-56° was obtained by salting it out of the aqueous phase with sodium chloride into the ether phase followed by separation, and evaporation of ether phase. Superimposability of the infrared spectrum of the recovered phenoxathiin(L) with that of the starting phenoxathiin(L) and the mixture melting point of the two solids showed that the compounds were identical.

In order to determine whether the 4,4'-diphenoxathiinyl ketone(LVI) could have been formed by a combination of 2 molecules of lithium 4-phenoxathiincarboxylate(LIV), a tetrahydrofuran solution of 9.2 g. of lithium 4-phenoxathiincarboxylate(LIV), prepared by adding 1 equivalent of a 15% n-butyllithium(XLI) in hexane solution to 1 equivalent of pure 4-phenoxathiincarboxylic acid(LII), was heated under reflux for 25 hr. On working up the mixture as described above, a quantitative yield of 4-phenoxathiincarboxylic acid (LII) was obtained. Superimposability of the infrared spectrum of 4-phenoxathiincarboxylic acid(LII) with that of an authentic sample prepared from the reaction of phenoxathiin(L) and n-butyllithium(XLI) and the mixture melting point of the two solids showed that the compounds were identical.

In order to determine that 4,4'-diphenoxathiinyl ketone(LVI) was formed by the reaction of 4-phenoxathiinyllithium(LI) with a limited amount of carbon dioxide, the following experiment was performed. A solution of 10.0 g. (0.05 mole) of phenoxathiin(L) in 100 ml. of anhydrous ether

was stirred for 20 hr. under a sealed nitrogen atmosphere with 21.3 g. (0.05 mole) of a 15.05% solution of n-butyllithium(XLI) in hexane. Carbon dioxide was then bubbled through the solution to give a yellow solid. The solvents were separated and evaporated to leave an oil as residue. The oil crystallized upon trituration with cold petroleum ether. The solid was heated with petroleum ether and isolated by filtration. The filtrate was allowed to evaporate to give 2.1 g. of starting phenoxathiin(L), m.p. 53-55°. The 2.3 g. of residue was recrystallized from acetone to give only traces of impure ketone(LVI). Evaporation of the acetone gave 1.5 g. of starting phenoxathiin(L), m.p. 52-54°.

Preparation of 4,4'-Diphenoxathiinyl Ketone(LVI). To 10.0 g. (0.05 mole) of 4-phenoxathiinyllithium(LI) - calculated on the basis of a 70% yield for monometalation -, in 100 ml. of anhydrous ether was added 8.3 g. (0.037 mole) of lithium 4-phenoxathiincarboxylate(LIV), prepared by adding 1 equivalent of n-butyllithium(XLI) to 1 equivalent of 4-phenoxathiincarboxylic acid(LII) in 200 ml. of anhydrous ether. The mixture was heated under reflux in a sealed nitrogen atmosphere for 20 hr. The reaction mixture was filtered, and the solvent was allowed to evaporated from the filtrate to give 1.0 g. (6.4%) of impure 4,4'-diphenoxathiinyl ketone(LVI), m.p. 153-164°. Recrystallization of the impure ketone from acetone gave 0.2 g. of pure compound, m.p. 163-166°. The residue from filtration of the reaction mixture was soluble in water and upon acidification with 5%

hydrochloric acid precipitated 4-phenoxathiincarboxylic acid(LII).

The 4,4'-diphenoxathiinyl ketone(LVI) prepared as above gave an infrared spectrum identical with that of the 4,4'-diphenoxathiinyl ketone(LVI) formed in the reaction of phenoxathiin(L) with 1 equivalent of n-butyllithium(LXI). Moreover, a mixture melting point of the two melted at 163-166°. The compound gave a positive 2,4-dinitrophenylhydrazine test in dimethyl sulfoxide.

Anal. Calcd. for $C_{25}H_{14}O_3S_2$: C, 70.41; H, 3.31. Found: C, 70.58; H, 3.57.

Infrared Spectrum (Model 137-B, No. 5525): 3000(sh, broad), 1660(m), 1600(m), 1480(m), 1450(sh), 1420(s), 1290(w), 1275(m), 1220(m, broad), 1160(w), 1130(w), 1120(w), 1075(m, broad), 1025(w), 985(s), 945(m), 940(w), 915(w), 890(sh), 875(s), 850(w, broad), 825(m), 810(m), 780(m), 750(s), 740(s), 730(s), 720(sh), 675(m).

Ultraviolet Spectrum: $\lambda_{max}^{CHCl_3}$ 279 (log ϵ 4.09), 345 (log ϵ 3.35).

Preparation of Glycine Ethyl Ester Hydrochloride (LXXV). A solution of 20.0 g. (0.27 mole) of glycine (LXIII) in 200 ml. of absolute alcohol was saturated with hydrogen chloride and the mixture was heated under reflux for 12 hr. On cooling the solution, a dark solid precipitated. Two recrystallizations of the solid from absolute ethyl alcohol gave 6.0 g. (15.9%) of pure glycine ethyl

ester hydrochloride(LXXV), m.p. 143–146°; Lit. (61), m.p. 142–145°.

Infrared Spectrum (Model 137–B, No. 1615): 3500(w), 3000(s, broad), 2700(w), 1740(s), 1590(m), 1550(m), 1500(s), 1450(m), 1410(s), 1380(s), 1250(s), 1140(m), 1100(m), 1050(s), 1000(m), 910(s), 860(s), 820(m), 720(w, broad).

Preparation of Glycine Ethyl Ester. A solution of 5.0 g. (0.036 mole) of glycine ethyl ester hydrochloride (LXXV) in 2.5 ml. of water was cooled to 0°C and 2.0 ml. of ether were added. To this was added 8 ml. of a 33% sodium hydroxide solution also at 0°, and the mixture was stirred for 0.25 hr. Potassium carbonate and barium oxide were then added thickening the water layer to a paste which was removed by filtration. After extracting the filtrate several times with ether, the combined ether extracts were placed on a flash evaporator, and the solvent was removed under reduced pressure to give 1.0 g. (27.2%) of pure glycine ethyl ester as residue.

Infrared Spectrum (Model 137–B, No. 1723): 3500(s), 3000(s), 1740(s), 1590(m), 1520(s), 1450(m), 1420(m), 1380(m), 1350(w), 1300(sh), 1250(sh), 1200(s, broad), 1120(w), 1100(m), 1060(m), 1030(m), 960(m), 880(w), 860(w).

Attempted Preparation of Ethyl N–(4–Phenoxathiincarbonyl)glycinate. A solution of 3.0 g. (12.3 mmoles) of 4–phenoxathiincarboxylic acid(LII) in 20 ml. of thionyl chloride was heated under reflux for 2 hr. Excess thionyl

chloride was removed by distillation to leave 2.8 g. (86.5%) of impure 4-phenoxathiincarboxylic acid chloride(LXII) as residue. Two recrystallizations of the crude product from petroleum ether gave 1.3 g. (40.3%) of pure 4-phenoxathiincarboxylic acid chloride(LXII), m.p. 79-81°. A Beilstein test was positive for halogen.

Infrared Spectrum (Model 137-B, No. 2768): 1770(s), 1590(m), 1570(m), 1470(m), 1420(s), 1275(m), 1220(m), 1210(w), 1180(m), 1160(m), 1120(m), 1095(w), 1020(w), 960(w), 940(s), 875(w), 850(w), 810(m), 790(m), 755(s), 730(m), 675(s).

To a solution of 2.5 g. (0.01 mole) of 4-phenoxathiincarboxylic acid chloride(LXII), as prepared above, in 25 ml. of anhydrous benzene was added 5 ml. of anhydrous pyridine. An immediate precipitation of the pyridinium salt resulted; the solid gave a positive Beilstein test for halogen and was soluble in water. To this mixture was added 1.0 g. (0.01 mole) of glycine ethyl ester, as prepared above, and on stirring for 4 hr. a clear solution resulted. A brown viscous liquid residue was left after removal of the solvent by flash distillation under reduced pressure. The residue was shaken with a water and ether mixture. The ether layer was separated, and 40 ml. of hexane were added to cause the precipitation of the product. On recrystallization only 4-phenoxathiincarboxylic acid(LII) was obtained. Superimposability of the infrared spectrum of the 4-phenoxathiincarboxylic acid(LII) with that of an authentic sample prepared from the reaction of phenoxathiin(L) and n-butyl-

lithium(XLI) and the mixture melting point of the two solids showed that the compounds were identical.

Preparation of N-(4-Phenoxathiincarbonyl)glycine (LXVI). A solution of 0.92 g. (12.3 mmoles) of glycine (LXIII), 9.8 g. (24.6 mmoles) of a 10% sodium hydroxide solution, and 2.8 g. (10.6 mmoles) of 4-phenoxathiincarboxylic acid chloride(LXII) was stirred for 0.25 hr. On standing a brown solid precipitated from the solution. The solid was removed by filtration and dissolved in 75 ml. of water. Acidification of the filtrate with 5% hydrochloric acid gave 2.4 g. of precipitate, m.p. 140-180°. After washing the solid several times with 100 ml. portions of ether to remove any starting 4-phenoxathiincarboxylic acid chloride(LXII) and the hydrolysis product(LII), the residue was recrystallized twice from methyl alcohol with Norit treatment to give 0.5 g. (15.7%) of N-(4-phenoxathiincarbonyl)glycine (LXV), m.p. 220.5-222.5°.

Anal.* Calcd. for $C_{15}H_{11}NOS_4$: C, 59.78; H, 3.68 Found: C, 59.58; H, 3.63.

Infrared Spectrum (Model 137-B, No. 1945): 3450(m), 3000(m), 1740(sh), 1720(s), 1620(s), 1520(s), 1470(m), 1420(s), 1310(w), 1275(m), 1225(s), 1160(w), 1130(w), 1100(m), 1095(w), 1020(w), 975(w), 950(w), 925(w), 890(w), 860(s, broad), 820(w), 810(w), 770(m), 755(s), 730(w), 720(w), 675(w).

Preparation of N-(4-Phenoxathiincarbonyl)-L(-)-proline (LXVI). A solution of 3.0 g. (11.4 mmoles) of 4-phenoxathiincarboxylic acid chloride(LXII), 1.31 g. (11.4 mmoles) of L(-)-proline(LXIV), and 23.0 g. (22.3 mmoles) of a 4% sodium hydroxide solution was stirred for 3 hr. and the product was precipitated by the addition of 1N hydrochloric acid. Recrystallization of the product was attempted from various solvents, but all attempts gave an oil on cooling. The oil from the attempted recrystallization with 95% ethyl alcohol was triturated with cold ligroin (66-75°) to give 0.31 g. (7.8%) of N-(4-phenoxathiincarbonyl)-L(-)-proline (LXVI), m.p. 110-114° (dec.).

Anal. Calcd. for $C_{18}H_{15}NO_4S$: C, 63.33; H, 4.43. Found: C, 61.45; H, 4.33.

Infrared Spectrum (Model 137-B, No. 6923): 3450(m), 1720(s), 1620(sh), 1600(s, broad), 1470(m), 1450(sh), 1420(s), 1275(m), 1250(m), 1220(m), 1130(sh), 1100(m), 1095(m), 1020(m), 975(m), 925(m), 825(w), 810(m), 755(s, broad), 700(m), 675(m).

Reaction of Phenoxathiin(L) with Two Equivalents of n-Butyllithium(XLI). Following a modification of the procedure of Gilman (41), a solution of 20.0 g. (0.1 mole) of phenoxathiin(L) in 200 ml. of anhydrous ether was treated with 84.7 g. (0.20 mole) of a 15.06% n-butyllithium(XLI) in hexane solution. The solution was heated under reflux for 44 hr. in a sealed nitrogen atmosphere. The reaction mixture was then poured onto a slurry of carbon dioxide in

ether. After all the solid carbon dioxide and ether had evaporated, the residue was pulverized, taken up in water, and acidified with 5% hydrochloric acid causing the precipitation of 23.2 g. of acidic product, m.p. 140–270°. The product was extracted with 1 l. of hot benzene to leave 11.2 g. of acidic residue, m.p. 220–280°. Evaporation of the benzene gave 10.8 g. (44.4%) of impure 4–phenoxathiincarboxylic acid(LII).

The 11.2 g. of acidic residue was dissolved in 10% sodium hydroxide, and the solution was acidified with glacial acetic acid to precipitate 4.0 g. of acidic product, m.p. 290–340°. The filtrate was acidified with 10% hydrochloric acid to yield 5.3 g. of acidic product, m.p. 225–240°.

The acidic compound precipitated with glacial acetic acid was recrystallized from 2–butanone and glacial acetic acid (1:1) to yield a product, m.p. 345–355°. Addition of water to the recrystallization medium yielded a solid, m.p. 220–240°.

All solids having melting points of less than 240° were combined and heated with glacial acetic acid and the mixtures were filtered. The residue gave a product, m.p. 340–352°. The filtrate on cooling deposited a solid, m.p. 250–270°. Addition of water caused further precipitation of a compound, m.p. 230–240°.

All solids of melting point less than 270° were combined and recrystallized from 95% ethyl alcohol to yield 1.0 g. (3.47%) of 4,6–phenoxathiindicarboxylic acid(LXVIIa),

m.p. 270–273°; Lit. (41), m.p. 266–267°.

All solids of melting point greater than 340° were combined and recrystallized from ethyl cellosolve to yield 1.2 g. (3.54%) of 1,6–phenoxathiindicarboxylic acid(LXVIIIa), m.p. 350–355° (dec.); Lit. (41), m.p. 351–353° (dec.).

This procedure was, and modifications of it were, carried out several times to obtain the two pure phenoxathiindicarboxylic acids, LXVIIa and LXVIIIa, in approximately the same yields as those reported above. One preparation gave on benzene extraction of the 4–phenoxathiincarboxylic acid(LII), a residue, m.p. 325–340°. Using the above procedure, separation yielded 1,6–phenoxathiindicarboxylic acid(LXVIIa), (3%).

Alternate procedures used to separate the dicarboxylic acids: Procedure A – During the preparations of the esters of 4,6–phenoxathiindicarboxylic acid(LXVIIa) and 1,6–phenoxathiindicarboxylic acid(LXVIIIa) by way of their acid chlorides, see below, it was found that 4,6–phenoxathiindicarboxylic acid(LXVIIa) underwent reaction at a faster rate with thionyl chloride than did 1,6–phenoxathiindicarboxylic acid(LXVIIIa). Accordingly, a separation using this reaction was tried. After removal of the 4–phenoxathiincarboxylic acid(LII) from the mixture of acids by way of benzene extraction in a Soxhlet extractor, the residue was treated with thionyl chloride. At 0.5 hr. intervals the reaction mixture was filtered and the insoluble residue was again treated with thionyl chloride. This procedure was repeated up to 3 hr. until all the dicarboxylic acids had

gone into solution. The filtrate at each interval was concentrated by flash distillation under reduced pressure, and the residue was stirred with a 10% sodium hydroxide solution. Acidification of the solutions with 5% hydrochloric acid gave the impure dicarboxylic acids. Recrystallization of the products of melting point less than 280° and those of more than 300° gave from 40.0 g. (0.20 mole) of phenoxathiin(L), 1.1 g. (1.9%) of 4,6-phenoxathiincarboxylic acid (LXVIIa), m.p. 270-273° and 2.1 g. (3.6%) of 1,6-phenoxathiindicarboxylic acid(LXVIIIa), m.p. 350-355° (dec.).

Procedure B - Since 4,6-phenoxathiindicarboxylic acid(LXVIIa) is more soluble in N-ethyl aniline than is 1,6-phenoxathiindicarboxylic acid(LXVIIIa), a separation using this property was attempted. After removal of the 4-phenoxathiincarboxylic acid(LII) as above, the residue was stirred with N-ethyl aniline. Impure 1,6-phenoxathiindicarboxylic acid(LXVIIIa) was isolated as a residue by filtration. Acidification of the N-ethyl aniline solution yielded impure 4,6-phenoxathiindicarboxylic acid(LXVIIa). Recrystallization of the impure 1,6-phenoxathiindicarboxylic acid(LXVIIIa) gave approximately a 9% yield of product, m.p. 345-350° (dec.). Recrystallization of the impure 4,6-phenoxathiindicarboxylic acid(LXVIIa) gave approximately a 4% yield of impure product, m.p. 250-265°, showing carboxylic acid bands as well as slight amide bands in the infrared spectrum.

Procedure C - The use of a higher reaction temperature was tried to get a larger ratio of dicarboxylic acids to monocarboxylic acid. Hexane was used in place of the ether as solvent to yield approximately the same ratio of

products as obtained by the main procedure.

Anal. Calcd. for $C_{14}H_8O_5S$ (4,6-phenoxathiindicarboxylic acid(LXVIIa): C, 58.33; H, 2.80. Found: C, 58.33; H, 2.49.

Infrared Spectrum (Model 137-B, No. 6458) - 4,6-phenoxathiindicarboxylic acid(LXVIIIa): 3400(m), 2900(m, broad), 1740(s), 1710(s), 1600(s), 1590(w), 1570(w), 1515(w), 1470(w), 1460(m), 1420(s), 1410(w), 1325(s), 1280(m), 1240(m), 1210(m), 1180(m), 1150(w), 1085(m), 980(w, broad), 940(m), 890(s), 850(w, broad), 810(s), 760(s), 720(s), 710(sh).

Infrared Spectrum (Model 137-B, No. 3676) - 1,6-phenoxathiindicarboxylic acid(LXVIIIa): 3400(m, broad), 2900(m, broad), 1670(s), 1650(sh), 1600(w), 1590(w), 1570(sh), 1515(sh), 1460(w), 1410(m), 1280(m), 1240(w), 1210(sh), 1160(m), 1140(m), 1080(w), 1025(m, broad), 980(m), 950(m), 890(m), 850(m), 810(s), 755(s), 750(s), 720(m), 700(s).

Reaction of Phenoxathiin(L) with Three Equivalents of n-Butyllithium(XLI). To 20.0 g. (0.1 mole) of phenoxathiin(L) dissolved in 100 ml. of anhydrous ether was added 126.9 g. (0.3 mole) of a 15.15% solution of n-butyllithium (XLI) in hexane, and the reaction mixture was allowed to stir at room temperature under a nitrogen atmosphere for 20 hr. Following carboxylation and acidification, as previously described, there was obtained 22.5 g. of acidic product. The acidic product was heated with several 250 ml.

portions of benzene and filtered until the filtrate was a pale yellow color. The residue weighed 5.4 g.

The filtrate was subjected to flash distillation under reduced pressure to leave a residue which on recrystallization from glacial acetic acid yielded 10.2 g. (41.8%) of 4-phenoxathiincarboxylic acid(LII), m.p. 167-168°. The infrared spectrum was superimposable with that of authentic 4-phenoxathiincarboxylic acid(LII) prepared by the reaction of phenoxathiin(L) with 1 equivalent of n-butyllithium(XLI).

The residue of 5.4 g. was heated under reflux with thionyl chloride for 3 hr. Excess thionyl chloride was removed by distillation to give 3.2 g. of product. This was added to 10 ml. of anhydrous pyridine and 15 ml. of absolute ethyl alcohol and the resulting solution was heated under reflux for 20 min. The solution was poured into 50 ml. of water and was allowed to stand for 2 days, during which time the precipitation of a white solid occurred. The solid isolated by filtration was 0.5 g. of residue, m.p. 106-109°. The filtrate was subjected to flash distillation under reduced pressure to leave a viscous oily residue. The oil was shaken with 5% sodium hydroxide and ether. The ether phase was separated and evaporated to give an impure product which when recrystallized from ethyl alcohol gave a trace amount of a compound, m.p. 59-62°, whose infrared spectrum was identical to that of diethyl 4,6-phenoxathiindicarboxylate (LXVIIc) prepared from pure 4,6-phenoxathiindicarboxylic acid chloride(LXXXVI), see below, and ethyl alcohol.

The 0.5 g. of residue, m.p. 106–109°, was recrystallized twice from ethyl alcohol and water to give an analytical sample of diethyl 1,6-phenoxathiindicarboxylate(LXVIIIc), m.p. 110.5–112°, whose infrared spectrum was identical to diethyl 1,6-phenoxathiindicarboxylate(LXVIIIc) prepared from 1,6-phenoxathiindicarboxylic acid chloride and ethyl alcohol.

Preparation of Dimethyl 4,6-Phenoxathiindicarboxylate (LXVIIb). To 1.0 g. (3.1 mmoles) of 4,6-phenoxathiindicarboxylic acid chloride(LXXXV), see below, was added 20 ml. of methyl alcohol and the solution was heated under reflux for 12 hr. The methyl alcohol was removed by flash distillation under reduced pressure to leave 0.60 g. of residue, m.p. 78–80°. Recrystallization of the solid residue from methyl alcohol and water gave 0.52 g. (52.7%) of dimethyl 4,6-phenoxathiindicarboxylate(LXVIIb), m.p. 81–82°; Lit. (41), m.p. 79–81°.

Anal. Calcd. for $C_{16}H_{12}O_5S$: C, 60.75; H, 3.80. Found: C, 60.48; H, 3.79.

Infrared Spectrum (Model 137-B, No. 6934): 3500(sh), 2900(w), 1730(s), 1710(w), 1590(m), 1570(w), 1460(w), 1420(s), 1280(w), 1250(m), 1220(m), 1200(m), 1190(sh), 1160(s), 1130(s), 1085(w), 1060(w), 985(s), 975(m), 890(m), 875(s), 850(m), 810(s), 760(s), 740(s), 735(sh), 720(w), 700(sh).

Ultraviolet Spectrum: $\lambda_{max}^{CHCl_3}$ 307(log ϵ 3.568).

The diethyl ester(LXVIIc), m.p. 59–62°, was also prepared by a similar procedure in 28.3% yield.

Infrared Spectrum (Model 137–B, No. 4716): 3400(w, broad), 2900(m), 1730(s), 1710(sh), 1600(w), 1590(w), 1450(sh), 1420(s), 1410(sh), 1400(sh), 1380(m), 1275(w, broad), 1220(m), 1210(s), 1180(sh), 1160(m), 1130(s), 1090(w), 1020(s), 985(sh), 940(w), 920(w), 875(w), 850(w, broad), 825(m), 800(sh), 770(s), 725(m).

Preparation of Dimethyl 1,6–Phenoxathiindicarboxylate(LXVIIIb). To 1.0 g. (3.46 mmoles) of 1,6–phenoxathiindicarboxylic acid(LXVIIa) was added 25 ml. of thionyl chloride, and the solution was heated under reflux for 1 hr. After cooling the solution to room temperature, the thionyl chloride was removed by evaporation under aspirator pressure to leave a green residue. The residue was taken up in 10 ml. of methyl alcohol and heated under reflux for 15 min. The solution was poured into ice water. On standing 0.8 g. of solid, m.p. 131–142°, precipitated from the solution. The solid was washed with a 5% sodium hydroxide sclution and recrystallized twice from absolute ethyl alcohol to give 0.5 g. (45.5%) of dimethyl 1,6–phenoxathiindicarboxylate (LXVIIb), m.p. 153–155°; Lit. (41), m.p. 150.5–151.5°.

Anal. Calcd. for $C_{16}H_{12}O_5S$: C, 60.75; H, 3.80. Found: C, 60.58; H, 3.65.

Infrared Spectrum (Model 137–B, No. 6901): 3500(sh), 3000(w), 2900(w), 1725(s), 1705(s), 1600(w), 1590(w), 1570(sh), 1515(sh), 1470(w), 1440(s), 1420(s), 1280(m),

1240(sh), 1220(w), 1190(m), 1150(m), 1110(m), 1080(w), 1050(w), 1010(s), 980(w, broad), 910(m), 850(m), 825(m), 800(m), 760(w), 740(w), 715(m), 705(sh).

Ultraviolet Spectrum: $\lambda_{max}^{CHCl_3}$ 333 (log ϵ 3.681).

Preparation of Diethyl 1,6-Phenoxathiindicarboxylate (XLVIIIc). To 0.9 g. (3.12 mmoles) of 1,6-phenoxathiindicarboxylic acid(XLVIIIa) was added 25 ml. of thionyl chloride, and the solution was heated under reflux for 4 hr. Thionyl chloride was removed by evaporation under reduced pressure to leave a green residue. The residue was dissolved in 10 ml. of absolute ethyl alcohol, and that solution was heated under reflux for 10 min. On pouring the solution into ice water, 0.7 g. solid, m.p. 94-99°, precipitated. Two recrystallizations of the solid from ethyl alcohol and water gave 0.3 g. (30.0%) of diethyl 1,6-phenoxathiindicarboxylate(XLVIIIc), m.p. 106-107°.

Anal. Calcd. for $C_{18}H_{16}O_5S$: C, 62.72; H, 4.69; 344.33 g./mole. Found: C, 62.81; H, 4.83; 362.11 g./mole.

Infrared Spectrum (Model 137-B, No. 3063): 2900(w), 1730(s), 1710(s), 1600(w), 1580(w), 1480(sh), 1420(s), 1400(sh), 1380(m), 1280(w), 1250(w), 1220(w), 1200(sh), 1190(w), 1160(m), 1130(m), 1090(w), 1060(w), 1020(s), 985(sh), 960(m), 925(sh), 875(w), 860(w), 825(m), 810(sh), 760(s), 735(sh), 715(w).

Attempted Preparation of 4-Hydroxymethylphenoxathiin(LXX). To a suspension of 0.38 g. (10 mmoles) of lithium aluminum hydride in 200 ml. of anhydrous tetrahydrofuran at -5° was added a solution of 1.29 g. (5 mmoles) of 4-phenoxathiincarboxylic acid(LII) in 200 ml. of anhydrous tetrahydrofuran, and the reaction mixture was allowed to warm to room temperature with stirring during 4 hr. Following the decomposition of excess lithium aluminum hydride with water and removal of the precipitated aluminum hydroxide, the tetrahydrofuran layer was separated, and the solvent was removed by evaporation to give a quantitative recovery of 4-phenoxathiincarboxylic acid(LII), m.p. 169-171°. Superimposability of the infrared spectrum of the recovered 4-phenoxathiincarboxylic acid(LII) with that of starting 4-phenoxathiincarboxylic acid(LII) and the mixture melting point of the two solids showed that the compounds were identical.

Preparation of 4-Hydroxymethylphenoxathiin(LXX). Procedure A - A solution of 1.5 g. (5.8 mmoles) of methyl 4-phenoxathiincarboxylate(LIII) in 200 ml. of anhydrous ether was added at room temperature over a period of 0.5 hr. to 1.5 g. (40 mmoles) of lithium aluminum hydride, and the mixture was stirred at room temperature for 3.5 hr. Excess lithium aluminum hydride was decomposed by the addition of water. The precipitated aluminum hydroxide was dissolved by the addition of 10% hydrochloric acid. The ether layer was

separated and allowed to evaporate to yield, after filtration, 0.27 g. of product, m.p. 75–82°. The crude solid was recrystallized from benzene with Norit treatment to give 0.1 g. (6.6%) of pure 4-hydroxymethylphenoxathiin(LXX), m.p. 92.5–94.0°.

Procedure B – To 3.1 g. (0.082 mole) of lithium aluminum hydride in 200 ml. of anhydrous ether was added over 1 hr. 5.0 g. (0.0204 mole) of 4-phenoxathiincarboxylic acid(LII) dissolved in 300 ml. of anhydrous ether. The reaction mixture was then stirred at room temperature for 17 hr. Excess lithium aluminum hydride was decomposed with water, and the precipitated aluminum hydroxide was dissolved with 10% hydrochloric acid. The organic compound was salted out of the aqueous phase by the addition of sodium chloride and taken up in ether. After separation of the ether layer and flash distillation under reduced pressure, a solid residue was obtained. This residue was stirred with a 5% sodium hydroxide solution and separated by filtration to leave a residue of 0.6 g. (12.8%) of impure 4-hydroxymethylphenoxathiin(LXX), m.p. 84–88°. Acidification of the filtrate with 5% hydrochloric acid gave 4-phenoxathiincarboxylic acid(LII). Superimposability of the infrared spectrum of the recovered acid(LII) with that of starting acid (LII) and the mixture melting point of the two solids showed that the compounds were identical.

Procedure C – This procedure is basically the same as procedure B except in the following:

(1) Tetrahydrofuran was used as a solvent, since 4-phenoxathiincarboxylic acid(LII) is more soluble in this solvent.

(2) The reaction mixture was heated under reflux for 3 to 20 hr.

(3) The reaction flask was cooled by an ice bath when excess lithium aluminum hydride was decomposed.

(4) Recrystallization was effected by extraction of the solid in a Soxhlet extractor with hot ligroin (66–75°) and cooling the receiver flask.

The average yield of pure compound, m.p. 89–91°, by this procedure was approximately 60%. The highest yield of pure compound, m.p. 89.5–91.5°, obtained was 73.6%.

Anal. Calcd. for $C_{13}H_{10}O_2S$: C, 67.79; H, 4.39. Found: C, 67.78; H, 4.49.

Infrared Spectrum (Model 137-B, No. 1946): 3300(m, broad), 3000(m), 1600(w), 1470(w), 1420(s), 1350(m), 1275(m), 1225(m), 1160(w), 1130(w), 1080(w), 1050(m), 1030(w), 960(m), 940(sh), 875(m), 810(m), 770(m), 755(s), 730(w), 720(w), 675(w).

Preparation of 4-Hydroxymethyl-6-Phenoxathiincarboxylic Acid(LXXI). To 10.0 g. (0.0434 mole) of 4-hydroxymethyl phenoxathiin(LXX) in 300 ml. of anhydrous ether was added 17.8 g. (0.043 mole) of a 15.15% solution of n-butyllithium(XLI) in hexane. A white solid, lithium alkoxide,

precipitated immediately. The mixture was stirred under a closed nitrogen atmosphere at room temperature for 6 hr. At this time a sample was removed. Carboxylation and acidification of the sample in the usual manner yielded only starting alcohol(LXX) (vide infrared spectrum). A second equivalent of n-butyllithium(XLI) was added and the reaction mixture was stirred at room temperature under a sealed nitrogen atmosphere for an additional 42 hr. The reaction mixture was poured onto a slurry of solid carbon dioxide in ether and was allowed to stand until all the solid carbon dioxide and solvents had evaporated. The residue was then stirred with a large volume of water, and the mixture was filtered. The filtrate was acidified with 5% hydrochloric acid to precipitate the impure product. Three recrystallizations of the solid from methyl alcohol using Norit at each recrystallization gave 6.5 g. (53.6%) of 4-hydroxymethyl-6-phenoxathiincarboxylic acid(LXXI), m.p. 229-231°.

The highest yield of pure product obtained by this procedure was 55%, m.p. 229-231°. Elemental analysis for sulfur was positive.

Anal.* Calcd. for $C_{14}H_{10}O_4S$: C, 61.31; H, 3.68. Found: C, 61.72; H, 3.91.

Infrared Spectrum (Model 337, No. 2273): 3300(s), 2945(m), 2500(m, broad), 1950(w, broad), 1685(s), 1600(w), 1450(sh), 1420(s), 1380(w), 1260(m), 1210(m), 1200(w), 1170(w), 1150(w), 1130(w), 1075(m), 1000(m), 970(m), 920(sh), 880(s), 850(w), 815(w), 805(w), 780(s), 760(w), 730(s), 720(s).

The phenylhydrazine derivative(LXXIX) melted at 226–228°.

Infrared Spectrum (Model 137–B, No. 3084): 3250(s, broad), 1950(sh), 1690(s), 1600(w), 1540(m), 1450(sh), 1410(s), 1400(sh), 1260(m), 1210(s), 1200(sh), 1170(w), 1150(w), 1130(w), 1075(m), 1000(m), 970(m), 920(sh), 880(s), 850(w), 815(w), 805(w), 780(sh), 760(s), 730(s), 720(sh).

The Reduction of 4–Hydroxymethyl–6–Phenoxathiincarboxylic Acid(LXXI). To a suspension of 1.5 g. (0.04 mole) of lithium aluminum hydride in 25 ml. of anhydrous tetrahydrofuran was added a solution of 2.74 g. (0.01 mole) of 4–hydroxymethyl–6–phenoxathiincarboxylic acid(LXXI) in 200 ml. of anhydrous tetrahydrofuran, and the mixture was heated under reflux for 23 hr. Excess lithium aluminum hydride was decomposed with water, and the precipitated aluminum hydroxide was dissolved with 5% hydrochloric acid. The organic compound was salted out of the aqueous phase, and the ether phase was separated and was evaporated to give 2.5 g. of residue, m.p. 102–135°. Recrystallization of the crude solid from carbon tetrachloride gave 1.1 g. (38.7%) of 4,6–dihydroxymethylphenoxathiin(LXXII), m.p. 138–140°. Superimposability of the infrared spectrum of 4,6–dihydroxymethylphenoxathiin(LXXII) with that of an authentic sample prepared from the reaction of lithium aluminum hydride with 4,6–phenoxathiindicarboxylic acid(LXVIIa) and the mixture melting point of the two solids showed that the compounds were identical. An analytical sample, prepared by repeated re–

crystallization from carbon tetrachloride, melted at 140–141.5°.

Anal.* Calcd. for $C_{14}H_{12}O_3S$: C, 64.60; H, 4.65. Found: C, 64.66; H, 4.72.

Infrared Spectrum (Model 137–B, No. 3613): 3460(m), 3375(m), 2950(w, broad), 1600(w), 1450(sh), 1420(s), 1350(m), 1260(m), 1210(m), 1170(m), 1150(w), 1130(w), 1080(s), 1060(s), 1020(m), 1010(m), 980(m), 960(w), 890(w), 870(m), 850(w), 810(m), 780(sh), 775(s), 760(s), 740(m), 720(m).

The Reduction of 4,6–Phenoxathiindicarboxylic Acid (LXVIIa). A solution of 1.0 g. (3.5 mmoles) of 4,6–phenoxathiindicarboxylic acid(LXVIIa) in 50 ml. of anhydrous tetrahydrofuran was added to a suspension of 0.7 g. (18 mmoles) of lithium aluminum hydride in 20 ml. of anhydrous tetrahydrofuran and the mixture was heated under reflux for 22 hr. Excess lithium aluminum hydride was decomposed with water, and the aluminum hydroxide which precipitated was dissolved by the addition of 5% hydrochloric acid. Sodium chloride was then added to the mixture in order to salt out the organic product from the aqueous phase. The mixture was extracted with ether and the ether phase was separated. Evaporation of the ether left 1.0 g. of a sticky residue. After washing with a 5% sodium hydroxide solution, the residue was crystallized from a 70% ethyl alcohol solution to give 0.1 g. (11.0%) of 4,6–dihydroxymethylphenoxathiin (LXXII), m.p. 138.5–140°. The superimposability of the

infrared spectrum of 4,6-dihydroxymethylphenoxathiin(LXXII) with that of an authentic sample prepared from the reaction of 4-hydroxymethyl-6-phenoxathiincarboxylic acid(LXXI) with lithium aluminum hydride and the mixture melting point of the two solids showed that the compounds were identical.

The Reduction of 1,6-Phenoxathiindicarboxylic Acid (LXVIIIa). A solution of 1.0 g. (3.5 mmoles) of 1,6-phenoxathiindicarboxylic acid(LXVIIIa) in 50 ml. of anhydrous tetrahydrofuran was added to a suspension of 1.0 g. (26 mmoles) of lithium aluminum hydride in 20 ml. of anhydrous tetrahydrofuran and the mixture was heated under reflux for 20 hr. On working up the reaction mixture as above, there was obtained 0.6 g. (66.0%) of 1,6-dihydroxymethylphenoxathiin(LXXIII), m.p. 152-154°, after recrystallization from 95% ethyl alcohol.

Anal.* Calcd. for $C_{14}H_{12}O_3S$: C, 64.60; H, 4.65. Found: C, 64.35; H, 4.56.

Infrared Spectrum (Model 137-B, No. 3669): 3250(m, broad), 2950(w, broad), 1600(w), 1450(sh), 1420(s), 1350(s), 1260(m), 1210(m), 1180(m), 1150(w), 1110(w), 1080(m), 1060(m), 980(w), 960(w), 890(w), 870(w), 850(m), 775(s), 720(m), 710(w), 675(m).

Preparation of 4-Chloromethyl-6-Phenoxathiincarboxylic Acid Chloride(LXXIV). To 4.0 g. (14.5 mmoles) of 4-hydroxymethyl-6-phenoxathiincarboxylic acid(LXXI) was added 25 ml. of thionyl chloride, and the solution was

heated under reflux for 45 min. The excess thionyl chloride was removed by flash distillation under reduced pressure to leave a green solid as residue. Following two recrystallizations from ligroin (110–115°) with Norit treatment, the crude solid gave 2.7 g. (61.4%) of 4–chloromethyl–6–phenoxathiincarboxylic acid chloride(LXXIV), m.p. 99–102°.

Anal. Calcd. for $C_{14}H_8Cl_2O_2S$: C, 54.03; H, 2.59. Found: C, 54.55; H, 2.69.

Infrared Spectrum (Model 337, No. 2278): 2900(w, broad), 1760(s), 1600(w), 1575(m), 1450(m), 1410(s), 1285(m), 1260(m), 1235(m), 1210(m), 1180(m), 1150(m), 1090(w), 1075(w), 975(sh), 940(s), 920(w), 890(w,sh), 870(m), 800(w), 790(w), 775(w), 725(s), 690(s).

Preparation of N,N'–(4–Methyl–6–Phenoxathiincarbonyl)–diglycine(LXXVI). To a solution of 0.48 g. (6.4 mmoles) of glycine(LXIII) in 6.3 ml. of 2N (12.6 mmoles) sodium hydroxide was added 1.0 g. (3.2 mmoles) of 4–chloromethyl–6–phenoxathiincarboxylic acid chloride(LXXIV) in 6 ml. of benzene, and the mixture was stirred for 3 hr. at the end of which time all the benzene had evaporated. The solution was then acidified with 2N hydrochloric acid causing the precipitation of 1.1 g. (88%) of impure N,N'–(4–methyl–6–phenoxathiincarbonyl)diglycine(LXXVI), m.p. 120–130° (dec.). Recrystallization of the crude product from 95% ethyl alcohol gave an oil which on cooling with petroleum ether gave the product (LXXVI), m.p. 140–147° (dec.). A negative Beilstein test indicated the absence of chlorine.

Anal. Calcd. for $C_{18}H_{16}N_2O_6S$: C, 55.65; H, 4.15. Found: C, 55.70; H, 3.71.

Infrared Spectrum (Model 137-B, No. 5399): 3500(m, broad), 2900(m), 1725(s), 1700(sh), 1675(sh), 1650(s), 1600(sh), 1450(sh), 1420(s), 1410(sh), 1285(sh), 1260(sh), 1210(s), 1180(w), 1160(w), 1150(w), 1090(m), 1025(m), 975(w), 940(m), 920(w), 890(s), 800(w), 790(w), 760(s), 740(s), 725(m).

Preparation of Ethyl N-(4-Chloromethyl-6-Phenoxathiincarbonyl)glycinate. Procedure A - To a solution of 0.5 g. (16 mmoles) of 4-chloromethyl-6-phenoxathiincarboxylic acid chloride(LXXIV) in 10 ml. of anhydrous benzene was added 0.4468 g. (32 mmoles) of glycine ethyl ester hydrochloride(LXXV), and the reaction mixture was heated under reflux for 20 hr. Filtration removed the starting amino acid ester hydrochloride(LXXV) as a residue. Evaporation of the filtrate followed by three recrystallizations of the resulting residue from methyl alcohol and water (5:1) gave 0.27 g. (45.0%) of Ethyl N-(4-chloromethyl-6-phenoxathiincarbonyl)glycinate(LXXVII), m.p. 109-115°. A positive Beilstein test indicated the presence of chlorine.

Procedure B - Heating 4-chloromethyl-6-phenoxathiincarboxylic acid chloride(LXXIV) and glycine ethyl ester hydrochloride(LXXV) with triethylamine gave only recovered starting materials.

Procedure C – To 1.54 g. (49 mmoles) of 4–chloromethyl–6–phenoxathiincarboxylic acid chloride(LXXIV) in 25 ml. of anhydrous benzene was added a mixture of 1.37 g. (98 mmoles) of glycine ethyl ester hydrochloride(LXXV) in 41.5 g. of 8% sodium bicarbonate and 25 ml. of benzene. The reaction was stirred for 4 hr., and the benzene layer was separated and concentrated to give 1.3 g. of residue. The residue was heated with benzene and petroleum ether, and the solution was filtered. Evaporation of the filtrate gave, after recrystallization from 95% ethyl alcohol, 1.44 g. (78.3%) of ethyl N–(4–chloromethyl–6–phenoxathiincarbonyl)–glycinate(LXXVII), m.p. 118–118.5°.

Anal. Calcd. for $C_{18}H_{16}ClNO_4S$: C, 57.22; H, 4.27. Found: C, 58.03; H, 4.19.

Infrared Spectrum (Model 337, No. 2271): 3250(s), 2900(w, broad), 1760(s), 1650(s), 1600(w), 1585(w), 1520(s), 1450(w), 1420(s), 1375(w), 1350(w), 1265(m), 1240(m), 1220(w), 1190(s), 1160(m), 1070(m), 1010(m), 970(m, broad), 930(w), 910(w), 870(m), 805(m), 780(w), 760(s), 730(sh), 720(s).

The infrared spectra of the products isolated from procedures A and C were identical.

Reaction of 4–Chloromethyl–6–Phenoxathiincarboxylic Acid Chloride(LXXIV) with Methyl Alcohol. To 0.5 g. (1.6 mmoles) of 4–chloromethyl–6–phenoxathiincarboxylic acid chloride(LXXIV) was added 50 ml. of methyl alcohol and the solution was heated under reflux for 1.5 hr. On pouring the

reaction mixture into ice water, a solid precipitated from solution. After recrystallization from 95% ethyl alcohol, there was obtained 0.2 g. (40.4%) of methyl 4-chloromethyl-6-phenoxathiincarboxylate(LXXIX), m.p. 96-98°. The presence of chlorine was indicated by a positive Beilstein test.

Anal. Calcd. for $C_{15}H_{11}ClNO_3S$: C, 58.72; H, 3.65. Found: C, 58.43; H, 3.27.

Infrared Spectrum (Model 137-B, No. 6904): 3500(w, broad), 2900(w, broad), 1730(s), 1710(sh), 1600(w), 1575(w), 1410(w), 1285(m), 1260(w), 1235(w), 1210(w), 1180(w), 1150(w), 1120(m), 1090(w), 975(w, broad), 890(sh), 870(m), 800(m), 775(sh), 760(s), 725(s).

Preparation of Activated Manganese Dioxide. A mixture of 111.0 g. of manganese sulfate monohydrate, 160 ml. of water, and 117 ml. of 40% sodium hydroxide was added to a hot solution of 96.0 g. of potassium permanganate in 600 ml. of water over a period of 45 min., and the resulting mixture was stirred for 3 hr. at room temperature. The manganese dioxide was collected by filtration and was washed several times with 200 ml. portions of water. After drying the solid, there was obtained a nearly quantitative yield of black manganese dioxide.

Preparation of 4-Formylphenoxathiin(LXXXII). To 1.0 g. (4.35 mmoles) of 4-hydroxymethylphenoxathiin(LXX) dissolved in 50 ml. of tetrahydrofuran was added 10 g. of activated manganese dioxide. The progress of the oxidation

was followed by the decrease in the 3300 cm.$^{-1}$ band due to the 4-formylphenoxathiin(LXXXII) being produced. After 3 hr. the oxidation was complete. The manganese dioxide was removed by filtration and the solvent from the filtrate was allowed to evaporate leaving a light brown residue. The residue was recrystallized from ligroin (66-75°) to give 8.2 g. (82.3%) of pure 4-formylphenoxathiin(LXXXII), m.p. 91.5-93.0°. The product gave a positive 2,4-dinitrophenylhydrazine test.

Anal. Calcd. for $C_{13}H_8O_2S$: C, 68.41; H, 3.54. Found: C, 68.34; H, 3.49.

Infrared Spectrum (Model 137-B, No. 4480): 2800(w), 1695(s), 1600(m), 1550(m), 1470(m), 1425(sh), 1410(s), 1395(s), 1275(m), 1250(s), 1225(m), 1210(w), 1175(m), 1130(m), 1080(w, broad), 1030(w, broad), 975(w, broad), 950(w, broad), 890(s), 875(m), 810(s), 780(s), 770(m), 755(w), 730(m), 675(sh).

Ultraviolet Spectrum: $\lambda_{max}^{CHCl_3}$ 275(log ϵ 3.89), 350(sh), 362.5(log ϵ 3.70).

Oxidation Reactions Leading to 4-Phenoxathiincarboxylic Acid(LII). Procedure A -- Oxidations of 4-hydroxymethylphenoxathiin(LXX) with commercial hypochlorite, Chlorox, with or without sodium hydroxide and in the presence or absence of a tetrahydrofuran solvent gave products with wide melting point ranges showing bands in the infrared spectra at 3300 cm.$^{-1}$ due to hydroxy and 1695 cm.$^{-1}$ due to

aldehyde. These products gave 2,4-dinitrophenylhydrazones in poor yields. All attempts to purify these products failed.

Procedure B -- Oxidations of 4-formylphenoxathiin (LXXXII) with silver oxide with or without chloroform as solvent gave only starting material after the reaction was allowed to stir at room temperature for several hours.

Procedure C - Heating a solution of 4-formylphenoxathiin(LXXXII) in the presence of oxygen gave only recovered starting material.

Procedure D - To 1.0 g. (4.35 mmoles) of 4-formylphenoxathiin(LXXXII) in 50 ml. of carbon tetrachloride was added a 10% solution of bromine in carbon tetrachloride until the solution retained a red color even after gentle heating. The carbon tetrachloride was removed by flash distillation under reduced pressure, and the solid residue was stirred with a 5% solution of sodium hydroxide. Filtration of the mixture and acidification of the filtrate with a 5% hydrochloric acid solution gave 0.11 g. (10.3%) of 4-phenoxathiincarboxylic acid(LII), m.p. 173-176°. Superimposability of the infrared spectrum of 4-phenoxathiincarboxylic acid(LII) with that of an authentic sample prepared by the reaction of phenoxathiin(L) with n-butyllithium (XLI) and the mixture melting point of the two solids showed that the compounds were identical.

Preparation of 4-Formyl-6-Phenoxathiincarboxylic Acid(LXXXIV). Procedure A - To 0.5 g. (1.82 mmoles) of 4-hydroxymethyl-6-phenoxathiincarboxylic acid(LXXI) was added 15 ml. of dimethyl sulfoxide, and the solution was heated under reflux for 2 hr. with oxygen bubbling through the solution. The solution was poured into 5 ml. of water causing the precipitation of an orange solid. The solid was then removed by filtration, and was washed several times with water to give a compound, m.p. 237-242°, which gave a positive 2,4-dinitrophenylhydrazine test. Recrystallization of the solid was effected by heating with ethyl alcohol and adding water until cloudiness persisted in the hot solution. This procedure gave 0.2 g. (40.5%) of 4-formyl-6-phenoxathiincarboxylic acid(LXXXIV), m.p. 242-244°.

The yield of LXXXIV could be increased to 66.7% by using a smaller amount of dimethyl sulfoxide and bubbling the oxygen through the solution until a solid began to settle out of solution and signs of charring of the solid were evident. At this time the reaction was terminated and worked up as described above.

Due to the inherent dangers of fire in this latter procedure, the former procedure was used with the concommitant decrease in yield.

Anal. Calcd. for $C_{14}H_8O_4S$: C, 61.76; H, 2.96. Found: C, 62.17; H, 2.85.

Infrared Spectrum (Model 337, No. 2386): 3325(w), 3050(w), 2900(w), 1720(sh), 1680(s), 1600(m), 1575(sh), 1555(m), 1510(w), 1450(s), 1425(s), 1390(s), 1270(sh),

1250(s), 1160(m), 1130(m), 1085(m), 1050(m), 1000(w), 975(w), 920(s), 880(s), 850(w), 810(m), 785(sh), 775(s), 755(s), 730(w), 710(s), 700(w).

Procedure B – Heating a mixture of manganese dioxide and 4--hydroxymethyl–6–phenoxathiincarboxylic acid(LXXI) resulted in the formation of a very impure product showing a carbonyl stretching absorption band due to aldehyde and acid as well as a hydroxyl stretching absorption band for alcohol and acid in the infrared spectrum. Attempts to purify the product were unsuccessful.

Preparation of 4,6--Phenoxathiindicarboxylic Acid–10–Oxide(LXXXV). To 5.0 g. (18.2 mmoles) of 4–hydroxymethyl–6–phenoxathiincarboxylic acid(LXXI) was added 50 ml. of concentrated nitric acid. The 4–hydroxymethyl–6--phenoxathiincarboxylic acid(LXXI) dissolved immediately with the evolution of brown–red nitrogen dioxide. A solid started to precipitate from the solution after 3 hr. standing at room temperature, and the precipitate continued to form during a period of 30 hr. The precipitate was removed by filtration, was washed with water, and was recrystallized from methyl alcohol to yield 4.3 g. (82.7%) of 4,6–phenoxathiindicarboxylic acid–10–oxide(LXXXV), m.p. 288–290°. An analytical sample prepared by repeated recrystallization from methyl alcohol, melted at 294–296°.

Anal. Calcd. for $C_{14}H_8O_6S$: C, 55.26; H, 2.65. Found: C, 55.58; H, 2.37.

Infrared Spectrum (Model 137-B, No. 6584): 2900(m, broad), 2400(w), 1720(sh), 1700(s), 1640(w), 1600(m), 1590(w), 1470(w), 1460(w), 1420(s), 1280(m), 1240(w), 1210(w), 1150(s), 1100(m), 1050(s), 940(m), 925(m), 890(s), 850(m), 825(m), 775(s), 735(sh), 725(s), 700(w, broad).

Heating the filtrate containing nitric acid at 70° for an additional 12 hr., cooling, and adding water did not yield further precipitate. Neutralization of the filtrate with a 40% sodium hydroxide solution caused the precipitation of tar.

In one experiment using this procedure the solution of concentrated nitric acid and 4-hydroxymethyl-6-phenoxathiincarboxylic acid(LXXI) was stirred with a magnetic stirrer. Precipitation occurred after 15 min. of stirring and was complete after 2 hr. The precipitate was removed by filtration, was washed with water, and was dried to give a solid, m.p. 278-285°. On the assumption that the solid was impure 4,6-phenoxathiindicarboxylic acid-10-oxide(LXXXV), it was stirred with 57% hydriodic acid to yield a product, m.p. 250-258°, whose infrared spectrum had a carbonyl band (broad) at 1700 $cm.^{-1}$. The compound has been assigned the structure of impure 4-formyl-6-phenoxathiincarboxylic acid (LXXXIV) based on the similarity of the infrared spectrum with that of authentic 4-formyl-6-phenoxathiincarboxylic acid(LXXXIV), prepared from the reaction of 4-hydroxymethyl-6-phenoxathiincarboxylic acid(LXXI) and dimethyl sulfoxide, a positive 2,4-dinitrophenylhydrazine test, and solubility in

a 5% sodium hydroxide solution. When the product of melting point 278--285° was placed in concentrated nitric acid and stirred for 12 hr., a compound, m.p. 265–287°, was obtained. This compound gave a positive 2,4–dinitrophenylhydrazine test, and the infrared spectrum showed a carbonyl absorption (broad) band at 1700 $cm.^{-1}$ and sulfoxide absorption at 1050 $cm.^{-1}$. The compound was accordingly assigned the structure of impure 4–formyl--6–phenoxathiincarboxylic acid–10–oxide (LXXX). All attempts to purify this compound by recrystallization from various solvents were unsuccessful. The product of melting point 250–258° was converted into 4,6–phenoxathiindicarboxylic acid(LXVIIa) by reaction with silver oxide. That the hydriodic acid did not convert 4,6–phenoxa–thiindicarboxylic acid–10–oxide(LXXXV) partially to 4–formyl–6–phenoxathiincarboxylic acid(LXXXIV) was proven by the fact that when 4,6–phenoxathiincarboxylic acid–10–oxide(LXXXV) was heated gently for 6 hr. with 57% hydriodic acid, only 4,6–phenoxathiindicarboxylic acid(LXVIIa) was obtained. Superimposability of the infrared spectrum of 4,6–phenoxa–thiindicarboxylic acid(LVIIa) with that of an authentic sample of (LVIIa) prepared by the reaction of phenoxathiin (L) with 2 equivalents of n–butyllithium(XLI) and a mixture melting point of the two solids showed that the compounds were identical.

Preparation of 4,6-Phenoxathiindicarboxylic Acid (LXVIIa). Procedure A - To 1.6 g. (5.53 mmoles) of 4,6-phenoxathiindicarboxylic acid-10-oxide(LXXXV) was added 15 ml. of a 57% hydriodic acid solution giving an immediate black coloration of the reaction mixture due to the presence of iodine. After stirring for 3 hr. the reaction mixture was poured into water, and was heated at the boiling temperature for approximately 0.5 hr. after the iodine coloration disappeared. On cooling the mixture, 1.3 g. (86.5%) of pure 4,6-phenoxathiindicarboxylic acid(LXVIIa), m.p. 269-271°, was obtained.

Superimposability of the infrared spectra of the above compound (LXVIIa) and the 4,6-phenoxathiindicarboxylic acid(LXVIIa) obtained from the reaction of phenoxathiin(L) with 2 equivalents of n-butyllithium(XLI) and mixture melting point of the two solids showed that the compounds were identical.

Anal. Calcd. for $C_{14}H_8O_5S$: C, 58.33; H, 2.80. Found: C, 58.73; H, 2.47.

The reduction of (LXXXV) by treatment with a 25% hydriodic acid solution with stirring for 24 hr. gave a product of melting point 262-282°. Recrystallization of the solid from various solvents failed to purify the compound. An infrared spectrum of the compound indicated that it was a mixture of 4,6-phenoxathiindicarboxylic acid(LXVIIa), hydroxy (OH) stretching vibration at 3400 $cm.^{-1}$, and 4,6-phenoxathiindicarboxylic acid-10-oxide(LXXXV), sulfoxide

(S→O) stretching vibration at 1050 cm.$^{-1}$. The latter compound(LXXXV) did not show the hydroxy (OH) stretching vibration at 3400 cm.$^{-1}$.

The following procedures removed entrapped iodine from 4,6-phenoxathiindicarboxylic acid(LXVIIa) in batches of less than 2 g., but, failed to do so in batches greater than 2 g. (1) The impure compound was heated with water until the iodine color disappeared. (2) The impure compound was dissolved in acetone, and the solution was allowed to evaporate.

The following procedures failed to remove entrapped iodine from 4,6-phenoxathiindicarboxylic acid(LXVIIa) in various sized batches: (1) recrystallization, (2) extraction of the iodine from the crude solid with ether, and (3) extraction of the iodine from the crude solid with carbon disulfide.

The best procedure found to remove entrapped iodine from 4,6-phenoxathiindicarboxylic acid(LXVIIa) in various sized batches was to stir the impure compound with a saturated aqueous solution of sodium thiosulfate, thus converting the iodine into iodide ions. The 4,6-phenoxathiindicarboxylic acid(LXVIIa) was not affected by this treatment and led to the recovery of pure 4,6-phenoxathiindicarboxylic acid(LXVIIa) in 94.3% yield.

Procedure B – To 0.41 g. (10.3 mmoles) of sodium hydroxide in 5 ml. of water was added with stirring 0.89 g. (5.25 mmole) of silver nitrate causing the precipitation of brown silver oxide. To the resulting mixture was added

0.561 g. (2.5 mmoles) of 4-formyl-6-phenoxathiincarboxylic acid(LXXXIV). After stirring the mixture for 4 hr. at room temperature, there was no evidence of formation of a silver mirror. Therefore, the mixture was heated gently for an additional 4 hr. A black colored product separated, and a silver mirror formed on the sides of the reaction vessel. The mixture was then filtered, and the residue was washed with warm water. A 5% hydrochloric acid solution was added to the cool filtrate to cause the precipitation of a solid which, after recrystallization from ethyl alcohol, and water, gave 0.55 g. (76.5%) of pure 4,6-phenoxathiin-dicarboxylic acid(LXVIIa), m.p. 167-169°. Superimposability of the infrared spectrum of this compound (LXVIIa) with that of the 4,6-phenoxathiindicarboxylic acid(LXVIIa) obtained by procedure A and mixture melting point of the two solids showed that they were identical.

Procedure C - To a suspension of silver oxide, prepared by mixing a solution of 2.4 g. (14.4 mmoles) of silver nitrate in 10 ml. of water with 1.2 g. (28.8 mmoles) of sodium hydroxide in 10 ml. of water, was added 1.0 g. (3.6 mmoles) of 4-hydroxymethyl-6-phenoxathiincarboxylic acid(LXXI). An additional 0.5 g. (12.5 mmoles) of sodium hydroxide was added after 1 hr. and the mixture was heated at 40° for 12 hr. The reaction mixture was then cooled and filtered, and the residue was washed with water. The filtrate was acidified with a 5% hydrochloric acid solution causing the precipitation of a solid, which on recrystal-lization from ethyl alcohol and water yielded 0.2 g. (21.1%)

of pure 4,6-phenoxathiindicarboxylic acid(LXVIIa), m.p. 268-271°. Superimposability of the infrared spectrum of this compound with that of the 4,6-phenoxathiindicarboxylic acid (LXVIIa) obtained by procedure A and the mixture melting point of the two solids showed that the compounds were identical.

Procedures Unsuccessful for the Preparation of 4,6-Phenoxathiindicarboxylic Acid(LXVIIa): (1) The oxidation of 4-hydroxymethyl-6-phenoxathiincarboxylic acid(LXXI) with chromic acid caused the oxidation of the sulfide linkage to the sulfoxide and gave an impure product. (2) The oxidation of 4-hydroxymethyl-6-phenoxathiincarboxylic acid(LXXI) with bromide in chloroform gave a very impure compound with an infrared spectrum showing bands characteristic of the starting material as well as bands characteristic of 4,6-phenoxathiindicarboxylic acid(LXVIIa). (3) The oxidation of 4-hydroxymethyl-6-phenoxathiincarboxylic acid(LXXI) by oxygen in boiling tetrahydrofuran gave an impure product which gave a positive 2,4-dinitrophenylhydrazine test. (4) The oxidation of 4-formyl-6-phenoxathiincarboxylic acid (LXXXIV) with bromine in carbontetrachloride gave only recovered starting material.

Preparation of 4,6-Phenoxathiindicarboxylic Acid Chloride(LXXXVI). To 2.0 g. (6.94 mmoles) of 4,6-phenoxathiindicarboxylic acid(LXVIIa) was added 20 ml. of thionyl chloride, and the solution was heated under reflux for 1.5 hr. The reaction mixture was cooled to room temperature, and

excess thionyl chloride was removed by evaporation under aspirator pressure to leave a light green residue. The residue was recrystallized from ligroin (66–75°) to give 2.0 g. (92.3%) of 4,6–phenoxathiindicarboxylic acid chloride(LXXXVI), m.p. 151–153°.

Anal. Calcd. for $C_{14}H_6Cl_2O_3S$: C, 51.71; H, 1.86. Found: C, 52.65; H, 1.86.

Infrared Spectrum (Model 337, No. 2395): 2900(w, broad), 1795(s), 1780(s), 1590(m), 1440(m), 1410(s), 1280(w), 1240(s), 1210(s), 1180(s), 1150(s), 1085(w), 980(sh), 950(s), 935(w), 865(s), 805(m), 785(s), 730(s), 690(m), 670(m), 655(w), 630(w).

An infrared spectrum of a chloroform solution of 4,6–phenoxathiindicarboxylic acid chloride(LXXXVI) showed a single band at 1765 $cm.^{-1}$(s) instead of the 1795 $cm.^{-1}$(s), 1780 $cm.^{-1}$(s) doublet.

Preparation of Diethyl N,N'–(4,6–Phenoxathiinbis–carbonyl)diglycinate(XCI). To 0.1 g. (0.31 mmoles) of 4,6–phenoxathiincarboxylic acid chloride(LXXXVI) in 100 ml. of benzene was added 2.80 g. (2.48 mmoles) of an 8% sodium bicarbonate solution and 0.087 g. (0.62 mmoles) of glycine ethyl ester hydrochloride(LXXV). The mixture was stirred for 12 hr. at room temperature. A white solid which had precipitated during the course of the reaction was removed by filtration. The benzene filtrate was separated, and the solution was concentrated to give a compound identical with

the precipitate. On recrystallization of the combined solids from 95% ethyl alcohol there was obtained 0.07 g. (49.3%) of diethyl N,N'-(4,6-phenoxathiinbiscarbonyl)diglycinate(XCI), m.p. 188-189.5°.

Anal. Calcd. for $C_{22}H_{22}N_2O_7S$: C, 57.62; H, 4.84. Found: C, 57.85; H, 4.95.

Infrared Spectrum (Model 337, No. 2385): 3360(sh), 3330(s), 2990(w), 2950(w), 1750(s), 1730(s), 1660(s), 1640(s), 1605(w), 1575(w), 1560(w), 1525(s), 1450(m), 1425(s), 1390(w), 1350(m), 1310(w), 1290(m), 1275(sh), 1265(s), 1240(s), 1230(s), 1210(s), 1190(s), 1160(s), 1140(w), 1120(w), 1090(s), 1075(w), 1030(s), 1010(w), 970(w), 940(m), 915(w), 890(m), 875(m), 870(w), 810(m), 800(m), 770(m), 760(s), 720(m), 700(w), 690(w), 650(s), 630(w).

Preparation of Dimethyl N,N'-(4,6-Phenoxathiinbis-carbonyl)-L(-)-dileucinate(XCII). Procedure A - To 0.45 g. (1.56 mmoles) of 4,6-phenoxathiindicarboxylic acid(LXVIIa) was added 20-ml. of thionyl chloride, and the solution was stirred for 4 hr. Excess thionyl chloride was removed by evaporation under aspirator pressure to give 4,6-phenoxa-thiindicarboxylic acid chloride(LXXXVI) as a yellow solid. The solid was dissolved in spectral-grade chloroform and added to 1 ml. of triethylamine and 0.61 g. (3.12 mmoles) of L(-)-leucine methyl ester hydrochloride(LXXXVII). The resulting solution was stirred for 48 hr. at room tempera-ture with no noticeable precipitation of triethylamine hydrochloride. The reaction mixture was heated under reflux

for an additional 5 hr. and was cooled and triethylamine hydrochloride precipitated from solution. The salt was removed by filtration, and the chloroform was allowed to evaporate to give a viscous oil which could not be solidified by standard procedures.

Infrared Spectrum (Model 137-B, No. 5235): 3300(s), 2900(s), 1740(s), 1710(w), 1650(s), 1600(w), 1550(sh), 1510(m), 1440(m), 1410(m), 1400(m), 1370(m), 1340(m), 1280(m), 1200(m, broad), 1160(m), 1140(sh), 1120(sh), 1090(m), 1030(s), 970(w), 925(w, broad), 900(w), 875(m), 805(m), 750(s).

Procedure B - To a mixture of 26 g. (24.6 mmoles) of an 8% sodium bicarbonate solution and 0.91 g. (6.16 mmoles) of L(-)-leucine methyl ester hydrochloride(LXXXVII) was added 1.0 g. (3.08 mmoles) of 4,6-phenoxathiindicarboxylic acid chloride(LXXXVI) in 50 ml. of benzene, and the solution was stirred for 24 hr. at room temperature. The benzene phase was separated and allowed to evaporate leaving a residual oil which could be solidified by shaking with cold petroleum ether. On addition of 5% sodium hydroxide to the resulting solid, it reverted back to an oil. The oil was separated and shaken with 5% hydrochloric acid. The oil was then separated and stirred with cold petroleum ether to give 0.9 g. (53.8%) of dimethyl N,N'-(4,6-phenoxathiinbiscarbonyl)-L(-)-dilucinate(XCII), m.p. 60-62°, $[\alpha]_D^{24}$ -30.6° (C 0.271, absolute ethyl alcohol).

Anal. Calcd. for $C_{28}H_{34}N_2O_7S$: C, 61.97; H, 6.32. Found: C, 61.60; H, 6.38.

Infrared Spectrum (Model 137-B, No. 7093): 3300(m), 2900(m), 1750(s), 1730(sh), 1670(s), 1640(s), 1600(w), 1550(sh), 1510(s, broad), 1470(sh), 1440(m), 1410(s), 1350(w, broad), 1325(sh), 1280(m), 1210(m, broad), 1160(m), 1140(sh), 1090(m), 1075(sh), 1030(m), 990(w), 970(w), 925(w), 900(w), 875(m), 830(m), 810(m), 760(s), 730(w, broad).

Ultraviolet Spectrum: λ_{max}^{EtOH} 303 mμ (log ε 3.740).

Preparation of Diethyl N,N'-(4,6-Phenoxathiinbis-carbonyl)-L(-)-diluc inate Monohydrate(CIII). To 0.18 g. (0.6 mmole) of 4,6-phenoxathiindicarboxylic acid chloride (LXXXVI) was added 0.235 g. (1.2 mmoles) of L(-)-leucine ethyl ester hydrochloride(LXXXI) in 10 ml. of benzene and 5.0 g. (4.8 mmole) of 8% sodium bicarbonate solution. The mixture was stirred for 15 hr. and the solvent was allowed to evaporate leaving an oil. After washing the oil with 5% hydrochloric acid and 5% sodium hydroxide solutions, it was taken up in hot absolute ethyl alcohol-petroleum ether, and the solution was allowed to evaporate to give diethyl N,N'-(4,6-phenoxathiinbiscarbonyl)-L(-)-diluc inate monohydrate (CIII) as a white solid, m.p. 45-47°.

Anal. Calcd. for $C_{30}H_{38}N_2O_7S$: C, 63.14; H, 6.71. Calcd. for $C_{30}H_{38}N_2O_7S \cdot H_2O$: C, 61.20; H, 6.86. Found: C, 61.22; H, 6.71.

Infrared Spectrum (Model 137-B, No. 6732), 3350(m, broad), 2900(m), 1740(s), 1710(w), 1650(m, broad), 1600(w), 1550(w), 1510(w, broad), 1440(w), 1410(w), 1350(sh), 1280(m), 1210(w, broad), 1160(m), 1140(sh), 1120(sh), 1090(m), 1030(m), 970(m), 950(w), 925(w), 900(w), 875(m), 810(m), 750(w), 725(s, broad).

Preparation of Dimethyl N,N'-(4,6-Phenoxathiinbis-carbonyl)-L(-)-diphenylalaninate(XCIII). To a mixture of 26 g. (24.6 mmoles) of an 8% sodium bicarbonate solution and 1.34 g. (6.1 mmoles) of L(-)-phenylalanine methyl ester hydrochloride(LXXXVIII) in 100 ml. of benzene was added 1.0 g. (3.08 mmoles) of 4,6-phenoxathiindicarboxylic acid chloride (LXXXVI) in 100 ml. of benzene, and the mixture was stirred for 24 hr. at room temperature. The benzene layer was separated and allowed to evaporate to leave an oil as residue. The oil solidified on stirring with petroleum ether in the cold. After treatment of the solid with 5% sodium hydroxide and with 5% hydrochloric acid there was obtained 1.2 g. (63.8%) of dimethyl N,N'-(4,6-phenoxathiinbiscarbonyl)-L(-)-diphenylalaninate(XCIII), m.p. 71-73°, $[\alpha]_D^{24}$ -37.5° (C 0.306, absolute ethyl alcohol).

Anal. Calcd. for $C_{34}H_{30}N_2O_7S$: C, 66.86; H, 4.95. Found: C, 66.75; H, 5.10.

Infrared Spectrum (Model 137-B, No. 7094): 3300(m, broad), 3000(w), 2900(w), 1750(s), 1730(sh), 1660(s, broad), 1600(w), 1550(sh), 1510(s, broad), 1440(m), 1410(s), 1350(sh), 1280(w), 1250(sh), 1210(m, broad), 1180(w),

1160(w), 1140(w), 1110(m), 1090(w), 1075(w), 1030(m), 990(w), 970(w), 925(w), 900(w), 875(m, broad), 860(w), 830(m), 810(m), 750(s, broad), 710(s).

Ultraviolet Spectrum: λ_{max}^{EtOH} 303 mμ (log ∈ 3.782).

An Attempt to Cyclize 4,6-Phenoxathiindicarboxylic Acid Chloride(LXXXVI) with Ethylene Glycol(XCIV). To a solution of 2.45 g. (7.5 mmoles) of 4,6-phenoxathiindicarboxylic chloride(LXXXVI) in 100 ml. of anhydrous benzene was added 0.47 g. (7.5 mmoles) of ethylene glycol(XCIV) during 0.5 hr. Stirring of the resulting solution was continued for 18 hr. The benzene was allowed to evaporate to leave a residue whose infrared spectrum shows a carbonyl absorption band at 1780 cm.$^{-1}$ ($\overset{O}{\overset{\|}{C}}$–Cl) and at 1720 cm.$^{-1}$ ($\overset{O}{\overset{\|}{C}}$–OR). The product was stirred with a 5% sodium hydroxide solution overnight, and the insoluble product was isolated by filtration to give 0.4 g. of residue having the following infrared spectrum (Model 137-B, No. 6906): 3400(s, broad), 1720(m), 1650(w), 1620(m), 1600(s), 1550(w), 1450(sh), 1410(s), 1400(w), 1280(sh), 1260(m), 1220(m), 1200(m), 1150(w), 1120(w), 1085(m), 1030(m, broad), 910(w), 890(w), 850(w), 810(s, broad), 770(s), 750(m), 725(w). The compound was assumed to be bis (2-hydroxyethyl) 4,6-phenoxathiindicarboxylate(CIV). Acidification of the filtrate yielded 4,6-phenoxathiindicarboxylic acid(LXVIIa), m.p. 263-268°. Superimposability of the infrared spectrum of 4,6-phenoxathiindicarboxylic acid(LXVIIa) with that of an authentic

sample of (LVIIa) prepared by the reaction of phenoxathiin (L) and 2 equivalents of n-butyllithium(XLI) and the mixture melting point of the two solids showed that the compounds were identical.

Reaction of 4,6-Phenoxathiindicarboxylic Acid Chloride(LXXXVI) with O-Phenylenediamine(XCV). A solution of 3.1 g. (2.83 mmoles) of O-phenylenediamine(XCV) in 50 ml. of benzene was added over 1 hr. to a mixture of 0.92 g. (2.83 mmoles) of 4,6-phenoxathiindicarboxylic acid chloride (LXXXVI), 2.40 g. (22.6 mmoles) of 8% sodium bicarbonate, and 50 ml. of benzene. On stirring for 5 hr. a solid precipitated from solution and was collected by filtration. The solid was washed with 5% sodium hydroxide and 5% hydrochloric acid to give a solid, m.p. 250-325°. The benzene filtrate was allowed to evaporate to give a solid, m.p. 250-330°. The two solids were combined and recrystallized from 95% ethyl alcohol and water to give 0.187 g. (18.2%) of pure compound, m.p. 325-326°, presumed to be the cyclic adduct (XCVI).

Anal. Calcd. for $C_{20}H_{12}N_2O_3S$: C, 66.65; H, 3.36; N, 7.77. Found: C, 66.62; H, 3.41; N, 8.05.

Infrared Spectrum (Model 137-B, No. 6713): 3400(m), 3300(m), 2900(m), 1690(s), 1650(s), 1600(m), 1590(w), 1550(s), 1500(m), 1450(s), 1420(s), 1340(s), 1290(s), 1260(s), 1240(s), 1210(w), 1195(w), 1160(m), 1120(w), 1090(w), 1080(w), 975(w), 955(m), 945(m), 935(m), 880(w), 875(m), 800(m), 790(m), 760(s), 740(s), 720(s), 705(m).

Preparation of Phenoxathiin-10-Oxide(XCVII). Procedure A - To a boiling solution of 15.0 g. (0.075 mole) of phenoxathiin(L) in 350 ml. of 95% ethyl alcohol was added 45 ml. of a 30% hydrogen peroxide solution. After heating under reflux on the steam bath for 3 hr., the solution was treated with an additional 30 ml. of 30% hydrogen peroxide. Heating was continued for an additional 9 hr., and the solution was allowed to cool overnight. The solution was reduced in volume to 100 ml., and hot water was added until a cloudiness persisted in the hot solution. On cooling the solution in an ice bath there was obtained 16.2 g. of a white solid, m.p. 148-156°. Recrystallization of the crude solid from ethyl alcohol and water gave 12.6 g. (77.7%) of phenoxathiin-10-oxide(XCVII), m.p. 154-156°; Lit. (52), m.p. 152-153°.

Procedure B - To a solution of 10 g. (0.05 mole) of phenoxathiin(L) in 200 ml. of glacial acetic acid was added 100 ml. of concentrated nitric acid and the resulting solution was warmed for 1.5 hr. On pouring the mixture into ice-water, there was obtained 8.2 g. (76.0%) of phenoxathiin-10-oxide(XCVII), m.p. 156-159°; Lit. (51), m.p. 151-154°.

Procedure C - To a boiling solution of 20 g. (0.10 mole) of phenoxathiin(L) in 300 ml. of 95% ethyl-alcohol was added 45 ml. of a 30% sulfuric acid solution, and the mixture was heated under reflux on the steam bath for 3 hr. Heating was continued for an additional 9 hr. after 30 ml.

of a 30% hydrogen peroxide solution was added. The solution was concentrated to 100 ml., and hot water was added to the hot solution until turbidity persisted. The precipitate was collected and recrystallized from methyl alcohol with Norit treatment to give 13.0 g. (60.2%) of phenoxathiin-10-oxide(XCVII), m.p. 155-158°; Lit. (52), m.p. 154-156°.

Anal. Calcd. for $C_{12}H_8O_2S$: C, 66.65; H, 3.73. Found: C, 67.17; H, 3.48.

Infrared Spectrum (Model 137-B, No. 3045): 2900(w, broad), 1580(s), 1480(w), 1450(s), 1430(s), 1270(m), 1220(m), 1180(w), 1160(w), 1130(m), 1085(w), 1070(w), 1040(m, broad), 940(w), 885(s), 875(s), 820(m), 770(s), 760(s), 730(w), 720(w), 675(m).

Ultraviolet Spectrum: $\lambda_{max}^{CHCl_3}$ 275(sh), 293(sh), 299(log ϵ 3.613).

The Reaction of Phenoxathiin-10-Oxide(XCVII) with Three Equivalents of n-Butyllithium(XLI). To a suspension of 21.6 g. (0.10 mole) of phenoxathiin-10-oxide(XCVII) in 300 ml. of anhydrous ether at -30° was added 126.8 g. (0.30 mole) of a 15.15% solution of n-butyllithium(XLI) in hexane, and the mixture was stirred in a sealed nitrogen atmosphere at -30° for 2 hr. The reaction mixture was then poured onto a slurry of solid carbon dioxide in ether and was allowed to stir in a hood until all the solid carbon dioxide and ether-hexane solvent had evaporated. The sticky residue weighed 45.0 g. This residue was then extracted for 75 hr. in a Soxhlet extractor with ligroin (66-75°).

Evaporation of the ligroin extracts left 15.1 g. of a liquid residue. Attempted distillation of the residue under reduced pressure caused extensive decomposition. Recrystallization of the pot residue of this attempted distillation from methyl alcohol gave 4.6 g. of starting phenoxathiin-10-oxide(XCVII).

The solid left behind in the Soxhlet thimble weighed 28.2 g. This solid was stirred with 150 ml. of water and the insoluble material was removed by filtration to give an additional 2.1 g. of phenoxathiin-10-oxide(XCVII).

Acidification of the filtrate with a 5% hydrochloric acid solution gave on filtration an aqueous filtrate and a sticky brown residue.

This filtrate was saturated with sodium chloride and extracted with ether and pentane. The extracts were separated and subjected to flash distillation under reduced pressure to leave 16.5 g. of a liquid residue, which on distillation gave 11.2 g. of valeric acid, b.p. 170-176°; Lit. (62), b.p. 184°. The infrared spectrum of this product was identical with authentic valeric acid.

The sticky brown residue was brought into solution by stirring with 100 ml. of warm benzene. On cooling the benzene solution 0.4 g. of 2,2'-dicarboxydiphenyl ether(C), m.p. 221-230°, precipitated from solution. Recrystallization from 2-butanone gave pure 2,2'-dicarboxydiphenyl ether(C), m.p. 226-228°; Lit. (53,54), m.p. 229-230°. Extraction of the benzene solution with a 5% sodium hydroxide solution and acidification of the aqueous extract with a 5% hydro-

chloric acid solution gave no acidic product.

Infrared Spectrum (Model 137-B, No. 3097) - 2,2'-Dicarboxydiphenyl Ether(C), m.p. 221-230°: 2900(s, broad), 2300(w), 2200(w), 1680(s), 1575(w), 1460(s), 1420(m), 1280(w), 1240(m), 1170(m), 1140(m), 1090(m), 1050(w), 950(s), 890(m), 875(w), 825(m), 805(sh), 760(s), 725(w), 695(m).

Infrared Spectrum (Model 137-B, No. 3253) - Valeric Acid: 3000(s, broad), 2700(w), 1720(s), 1470(m), 1410(m), 1390(w), 1280(m, broad), 1200(m, broad), 1100(m), 1050(w, broad), 940(s), 830(w, broad), 750(m).

Preparation of D(+)-Percamphoric Acid(CIV). To 200 ml. of water at 0° was added, with vigorous stirring and with cooling during 0.25 hr., 4.3 g. (0.056 mole) of sodium peroxide. A solution of 10 g. (0.05 mole) of D(+)-camphoric anhydride was then added to this solution during 1 hr. The mixture was stirred for 1 hr., and then the ether layer was separated and replaced with 200 ml. of fresh cold ether. The mixture was then acidified with 25 ml. of cold 6N sulfuric acid and was stirred for approximately 1 min. The ether layer was separated, was shaken with 100 ml. of a saturated ammonium sulfate solution to remove any free hydrogen peroxide, and was dried overnight at 0° with sodium sulfate. The solvent was removed by flash distillation at reduced pressure leaving 8.0 g. of a mixture consisting of 51.3% D(+)-percamphoric acid(CIV), as a viscous oil, $[\alpha]_D^{26}$

(+) 59.4°; Lit. (59), $[\alpha]_D^{25}$ (+) 55.1° (C 5.0, chloroform).

Cold petroleum ether failed to cause the product to solidify. The D(+)-percamphoric acid(CIV) was dried and stored in a vacuum desicator over concentrated sulfuric acid.

The peracid content of the 8.0 g. product was determined to be 51.3% by titration of solutions of 0.4N acetic acid, potassium iodide and 0.20 g. samples of the impure peracid(CIV) with 0.0207N sodium thiosulfate.

Repetition of this procedure gave a mixture consisting of 29.56% D(+)-percamphoric acid(CIV) as a solid.

The low yield of D(+)-percamphoric acid(CIV) apparently results on long periods of standing. This was evidenced by a decrease in the percent of D(+)-percamphoric acid(CIV) from 51.3% to 5.5% on storing in a vacuum desicator over concentrated sulfuric acid for 2 weeks.

Infrared Spectrum (Model 137-B, No. 6969): 3200(s, broad), 2950(s), 1720(sh), 1700(s), 1500(sh), 1480(sh), 1470(s), 1400(sh), 1380(m), 1320(w), 1280(w), 1240(w), 1200(w), 1150(w, broad), 1120(w), 1045(m), 875(m, broad), 800(m, broad), 760(m).

Reaction of D(+)-Percamphoric Acid(CIV) with Dimethyl 1,6-Phenoxathiindicarboxylate(LXVIIIC). Procedure A - A mixture of 0.709 g. (2.24 mmoles) of dimethyl 1,6-phenoxathiindicarboxylate(LXVIIIC) and 3.28 g. (4.48 mmoles) of 29.56% D(+)-percamphoric acid(CIV) in 150 ml. of chloroform was stirred for two days at -12°. An aliquot was removed

and tested for the presence of D(+)-percamphoric acid(CIV) by the addition of small amounts of potassium iodide. An immediate iodine coloration indicated the presence of D(+)-percamphoric acid(CIV). After stirring an additional two days at 30°, the solution still gave a positive potassium iodide test. After stirring for still an additional two days at room temperature, the potassium iodide test on an aliquot of the solution gave only a trace of iodine coloration. A solid, which had settled out of solution during the reaction, was removed by filtration and was found to be D(+)-camphoric acid, based on its solubility in 5% sodium hydroxide and superimposability of its infrared spectrum on the infrared spectrum of an authentic sample. The filtrate was allowed to evaporate to leave 1.143 g. of solid residue. The weight of the residue—higher than theoretical—indicated that it contained some acid impurity and so it was stirred with 5% sodium hydroxide. The undissolved solid was removed by filtration to leave 0.469 g. (60.3%) of dimethyl 1,6-phenoxathiindicarboxylate-10-oxide(CII), m.p. 146-149°. An analytical sample prepared by two recrystallizations from 95% ethyl alcohol, gave a melting point of 149-151°.

The filtrate from filtration of the sodium hydroxide mixture was acidified to yield D(+)-camphoric acid, as shown by superimposability of its infrared spectrum with that of authentic sample.

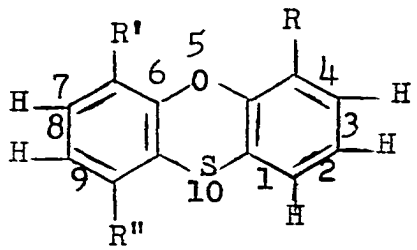
Anal. Calcd. for $C_{16}H_{12}O_6S$: C, 55.17; H, 3.45. Found: C, 55.56; H, 3.42.

Infrared Spectrum (Model 137-B, No. 7321): 3400(w), 2950(w), 1740(s), 1610(m), 1580(m), 1475(sh), 1430(s), 1340(m), 1320(sh), 1290(w), 1275(w), 1240(w), 1220(w), 1210(w), 1180(sh), 1160(m), 1140(m), 1090(w), 1075(w), 1005(m), 980(w), 940(m), 865(m), 855(m), 830(m), 815(m), 765(s), 755(w), 740(m), 720(w).

Procedure B — Repetition of Procedure A with 1.0 g. (2.38 mmoles) of 51.3% D(+)-percamphoric acid(CIV) and 0.5 g. (1.58 mmoles) of dimethyl 4,6-phenoxathiindicarboxylate(LXVIIIC) at 0° for 24 hr. gave an impure product, m.p. 126-128°. On treating an aliquot of the product with potassium iodide and acetic acid, a faint iodine coloration was noticed. No optical activity was observed for this product. Recrystallization of the solid gave impure dimethyl 4,6-phenoxathiindicarboxylate-10-dioxide(CII), m.p. 143-145°. Superimposability of the infrared spectrum of this compound (CII) with that of dimethyl 4,6-phenoxathiindicarboxylate-10-dioxide(CII) prepared in procedure A showed that the two solids were identical compounds.

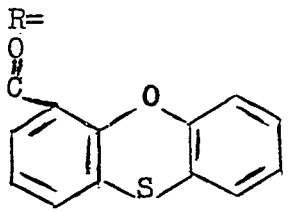
TABLE I

Nuclear Magnetic Resonance Spectra of Substituted Phenoxathiin



Substituent	Solvent	Chemical Shift (P.P.M.)--from TMS	Number of Hydrogens	J_{HH}(c.p.s.)	Assignment
R=H R'=H R"=H	CCl_4	6.94(singlet)			Aromatic
O ‖ R=C—OH		7.30(complex)	6		Aromatic
R'=H	DMSO	7.72(Quartet)*	1	ortho – 8.0 meta – 2.5	Position 3
R"=H					
O (a) ‖ R=C—H		7.11(complex)	6		Aromatic
R'=H	CCl_4	7.65(Quartet)*	1	ortho – 7.5 meta – 2.5	Position 3

Substituent	Solvent	Chemical Shift (P.P.M.)-from TMS	Number of Hydrogens	J_{HH}(c.p.s.)	Assignment
R"=H		10.61(singlet)	1		(a)
(b) (a) R=CH_2–OH		2.57(singlet)	1		(a)
R'=H	$DCCl_3$	4.65(singlet)	2		(b)
R"=H		6.88(complex)	7		Aromatic
O ‖ R=C–Cl		4.74(singlet)	2		(a)
(a) R'=CH_2–Cl	CCl_4	7.14(complex)	5		Aromatic
R"=H		7.85(Quartet)*	1	ortho – 7.5 meta – 2.5	Position 3
O (e) (c) ‖ R=C–NH–CH_2–		1.32(triplet)	3	a,b – 6.5	(a)
O (b) (a) ‖ C–O–CH_2–CH_3		4.24(Quartet)	2	b,a – 6.5	(b)
(d) R'=CH_2–Cl	CCl_4	4.15(doublet)	2	c,e – 3.5	(c)
		4.74(singlet)	2		(d)

Substituent	Solvent	Chemical Shift (P.P.M.)-from TMS	Number of Hydrogens	J_{HH}(c.p.s.)	Assignment
R"=H		7.05(complex)	5		Aromatic
		7.79(Quartet)*	2	ortho – 6.5 meta – 3.0	Position 3;(e)
(c) (d) R=CH_2–OH		4.54(doublet)	2	a,b – 3.5	(a)
R'=H	DMSO	4.75(doublet)	2	c,d – 3.5	(b)
(a) (b) R"=CH_2–OH		5.30(broad)*	2		(c); (d)
		7.25(complex)	6		Aromatic
O (a) R=C–OCH_3		4.03(singlet)	6		(a); (b)
R'=H	$DCCl_3$	7.18(complex)	4		Aromatic
O (b) R"=C–OCH_3		7.71(Quartet)*	2	poorly resolved	Positions 3,8
R= 		5.75(Quartet)*	2	ortho – 7.5 meta – 2.5	Positions 6,6'

Substituent	Solvent	Chemical Shift (P.P.M.)—from TMS	Number of Hydrogens	J_{HH}(c.p.s.)	Assignment
R'=H	$DCCl_3$	6.97(complex)	12		
R"=H		7.47(Quartet)*	2	ortho – 6.5 meta – 2.5	Positions 3,3'
(a) (b) R=CH_2—OH		4.65(singlet)	4		(a); (c)
(c) (d) R'=CH_2—OH	DMSO	5.47(broad)	2		(b); (d)
R"=H		7.22(complex)	2		Aromatic
O ‖ R=C—OH		7.46(complex)	6		Aromatic
O (a) ‖ R'=C—H	DMSO	10.78(singlet)	1		(a)
R"=H					
O ‖ R=C—OH		4.78(singlet)	2		(a)
(a) R'=CH_2—OH	DMSO	7.40(complex)	6		Aromatic
R"=H					

Substituent	Solvent	Chemical Shift (P.P.M.)—from TMS	Number of Hydrogens	J_{HH}(c.p.s.)	Assignment
R=R'=		1.32(triplet)	6	a,b – 6.5	(a)
O (d) (c) ‖ C–NH–CH_2–		4.25(Quartet)	4	b,a – 6.5	(b)
O (b) (a) ‖ C–O–CH_2–CH_3	$DCCl_3$	4.13(doublet)	4	c,b – 3.5	(c)
R"=H		7.17(complex)	4		Aromatic
		7.69(Quartet)*	4	ortho – 6.5 meta – 3.5	Position 3;(d)
O ‖ R=C–OH O ‖ R'=C–OH R"=H	DMSO	7.38(complex)			Aromatic
R=H		7.46(complex)	6		Aromatic
R'=H	$DCCl_3$	7.89(Quartet)*	2	ortho – 8.5 meta – 2.0	Positions 1,9

Substituent	Solvent	Chemical Shift (P.P.M.)—From TMS	Number of Hydrogens	J_{HH}(c.p.s.)	Assignment
R"=H oxide					
O (a) ‖ R=C—OH		7.60(triplet)	2	ortho – 7.5	Positions 3,7
O (b) ‖ R'=C—OH	DMSO	8.26(complex)	4		Aromatic
R"=H oxide		11.00(singlet)	2		(a),(b)

*, with fine splitting

SUMMARY

A method of synthesis of actinomycin analogs was developed in which the heterocyclic system of the natural antibiotic was substituted by the commercially available, tricyclic, and biologically-active ring system of phenoxathiin (L). In order to accomplish the synthesis of analogs of the heteroaroyl portion of actinomycin, the systhesis of 4,6-phenoxathiindicarboxylic acid (LXVIIa) was required. Metalation reactions on phenoxathiin (L) with n-butyllithium (XLI) varying not only the relative molar ratio of reactants, but also the temperature of the reaction were found to give low yields of 4,6-phenoxathiindicarboxylic acid (LXVIIa). A better procedure was therefore sought to obtain the desired dicarboxylic acid, LXVIIa.

A satisfactory synthesis of LXVIIa was found using as an intermediate the product of monometalation and carboxylation of phenoxathiin (L), 4-phenoxathiincarboxylic acid (LII). The acid, LII, was reduced to 4-hydroxymethylphenoxathiin (LXX) with lithium aluminum hydride. The alcohol, LXX, was metalated with n-butyllithium (XLI) and carboxylated to give 4-hydroxymethyl-6-phenoxathiincarboxylic acid (LXXI). Oxidation of LXXI with nitric acid to 4,6-phenoxathiindicarboxylic acid-10-oxide (LXXXV) followed by reduction with hydriodic acid readily gave the desired 4,6-phenoxathiindicarboxylic acid (LXVIIa).

The accomplishment of the desired synthesis of phenoxathiin analogs of actinomycin produced several additional small problems for investigation. (1) Oxidation reactions on 4-hydroxymethylphenoxathiin (LXX) and 4-hydroxymethyl-6-phenoxathiincarboxylic acid (LXXI) proved to be unusual and were investigated. (2) 4,4'-Diphenoxathiinyl ketone (LVI) was found to be formed in the metalation of phenoxathiin (L), and its structure and mode of formation were investigated.

Due mainly to the availability of the compounds, several additional syntheses were attempted. (1) The synthesis of a cyclic adduct from the reaction of 4,6-phenoxathiindicarboxylic acid chloride (LXXXVI) and o-phenylenediamine (XCV) was examined. (2) The asymmetric oxidation of dimethyl 1,6-phenoxathiindicarboxylate (LXVIIIb) to the corresponding sulfoxide was attempted. (3) The metalation of phenoxathiin-10-oxide (XCVII) was investigated as a method of preparation of 1-phenoxathiincarboxylic acid (XCVII).

The nuclear magnetic resonance spectra of the phenoxathiin derivatives were determined. These spectra appeared to indicate information about the rotational conformations of the ketone LVI and suggest differences in the hydrogen bonding of 4,6-dihydroxymethylphenoxathiin (LXXII) and 1,6-dihydroxymethylphenoxathiin (LXXII).

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